INVESTIGATIONS OF THE PHYSIOLOGY AND GENETICS OF THE NEW ZEALAND CONIFERS: RIMU, KAHIKATEA AND TOTARA

A thesis

submitted in partial fulfilment

of the requirements for the Degree

of

Doctor of Philosophy

in the

University of Canterbury

by

Barbara J. Hawkins

University of Canterbury

1988

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Page

Page

LIST OF FIGURES

LIST OF PLATES

Page

LIST OF APPENDICES

The forest is a peculiar organism of unlimited kindness and benevolence that makes no demands for its sustenance, and extends generously the products of its life activity. It affords protection to all beings, offering shade even to the axeman who destroys it.

Gautama Buddha

ABSTRACT

Several experiments were undertaken to examine some of the physiological and genetic factors which affect the distribution of rimu, kahikatea and totara, and which contribute to the overall geographic dominance of rimu in New Zealand.

A nutrition experiment, examining the growth of several provenances of each of the three species at six levels of nutrient supply, was carried out to explore species' differences in the demand for and utilization of nutrients. This work indicated that there is some differentiation among rimu, kahikatea and totara in their ability to utilize and respond to nutrients. Rimu appeared to have a greater ability to take up and store nutrients, while kahikatea and totara were more responsive to improved nutrition.

A second nutrition experiment repeated three of the treatments in the first experiment, with half of the seedlings in each nutrient treatment inoculated with mycorrhizas. The results of this experiment showed there to be a significant improvement in the growth of all species due to mycorrhizal infection, with rimu and kahikatea being more mycotrophic than totara. Growth was improved in all nutrient treatments, and this appeared to be due primarily to the enhanced uptake of phosphorous and, to a lesser extent, nitrogen by mycorrhizal fungi.

A germination experiment and two controlled environment experiments, germinating seeds and growing seedlings of the three species at different temperatures, were conducted to discover the optimum temperatures for germination, growth and net photosynthesis. The temperatures producing maximum rates for these parameters (around 27^{o}C) were much higher than anticipated for temperate species, and a third controlled environment experiment was then carried out with a subalpine podocarp species. The purpose of that

experiment was to examine the optimum growing temperature of a species which must have been under relatively severe selection pressures to adapt to its environment.

Snow totara, the subalpine species, had the same optimum temperature for growth and net photosynthesis ($27^{o}C$) as did the podocarp species from lower elevations. It is suggested that the high optimum temperatures for growth evolved in the New Zealand species during the subtropical conditions of the Miocene, and have been retained as a relic trait to the present. This would imply that selection pressures since that time have been acting upon attributes other than growth rate.

If selection pressures have not been acting upon growth rates in the recent past, significant provenance differentiation with respect to optimum growing temperature would not be expected. An experiment growing seedlings of seven totara provenances in two temperature regimes was undertaken to discover whether optimum growing temperature was correlated with the climate of seed origin. No such correlation could be found.

Isozyme analysis of seed of several populations of rimu, kahikatea and four species of Podocarpus was carried out to study the amount and partitioning of genetic variation within and between the species. Results from this work indicated that all species have lower levels of genetic variation than most northern hemisphere conifers. Rimu and kahikatea are also significantly less variable than the Podocarpus species. It is suggested that variability in the New Zealand species may have been lost through genetic drift in restricted populations during the cold periods of the glacial/interglacial cycles of the last two million years. The differences between the species may be due to their different chromosome numbers and the greater number of species in the genus Podocarpus which hybridise and thus maintain variability.

It appeared from the isozyme analysis, that some of the genetic variation within the podocarp species may be associated with environmental selection pressures. Since it seems that selection for growth rate has not been significant, selection for cold tolerance may have been important through the periods of Pleistocene glaciation. An experiment was undertaken to explore the frost tolerance of several provenances of rimu, kahikatea and totara to test whether provenance differentiation has occurred for this attribute. The frost tolerance of the eight totara provenances appeared to be correlated with their climate of origin, but the results for rimu and kahikatea were not conclusive.

While the distribution and overall dominance patterns of rimu, kahikatea and totara were not well explained by these experiments, an insight was gained into their evolutionary history and into the selection pressures that have shaped the species today. It was also demonstrated that the sequence of geologic and climatic events which resulted in a particular combination of selection pressures unique to New Zealand, have set this country's coniferous species apart in terms of physiology and genetic variation from most other conifer species of the world.

CHAPTER I

INTRODUCTION

The members of the Podocarpaceae are the most primitive of all gymnosperms (Sporne, 1965). The Stachycarpus section of this family is an extremely primitive group of conifers, while the Dacrycarpus section, to which rimu, kahikatea and totara belong, is somewhat more advanced (Sporne, 1965) (see Appendix A for scientific names of all species).

Early ancestors of today's podocarps were abundant in prehistoric forests over 200 million years ago (Miller, 1977). There is some debate as to when the present-day species of podocarps arose; but generally it is held that close relatives of rimu and kahikatea had evolved by the early Paleocene, 55 to 65 million years ago, whereas totara can only be traced back to the upper Miocene, 15 million years ago (Salmon, 1980).

Little is known of the physiology or genetic composition of the members of the Podocarpaceae in New Zealand. As they are such slow-growing species with lifespans of 700 years for rimu, 800 years for kahikatea and up to 1000 years for totara (Beveridge, 1983), forestry research efforts have mainly concentrated on faster growing, exotic species. Broad conclusions about the native species have been drawn from ecological studies and general observations; but work investigating the physiological attributes of the trees in controlled environments is limited.

Rimu is the most widely distributed and abundant of all New Zealand native forest trees (Franklin, 1968). It occurs from sea level to 950 m in the central North Island, to 600 m in the central South Island, and to 300-400 m in Fiordland and Stewart Island. Rimu is found on all but the very driest or wettest soils, but will not grow where the

snow lies for more than a few days at a time (Franklin, 1968). Once established, rimu is tolerant of a wide range of sites, soils and climate, but seedlings will only grow where the radicles can quickly penetrate to mineral soil or a continually moist substratum (Beveridge, 1983), and where they are sheltered from wind (Forest Research Institute, 1980). Young trees are very shade tolerant and may persist beneath a canopy for decades until a gap is opened overhead.

Kahikatea has a much more restricted range than rimu. It occurs from sea level to 700 m on pumice soils in the North Island, but is confined to valley bottoms and swamps in the South Island (Beveridge, 1983). This species is not as shade tolerant as rimu and prefers moist, fertile sites with full overhead light (Cameron, 1960). Under such conditions, kahikatea will grow 50 percent faster than rimu (Cameron, 1960), and this species grows to be the tallest of the native trees (Metcalf, 1972).

Totara is more xerophytic than rimu and is more able to occupy dry or exposed positions (Cockayne, 1928). It is the most drought resistant of the three species, but is the least shade tolerant (Forest Research Institute, 1980). This species is common in lowland, mountain and lower subalpine forests throughout the North and South Islands from sea level to 500 m. It may occur up to 700 m elevation, but at these altitudes it is generally replaced by the closely related Hall's totara (Metcalf, 1972). Hall's totara is considered to be more shade and frost tolerant than totara (Cameron, 1960), but these two species and other species and varieties of Podocarpus hybridise readily (Poole and Adams, 1980) which can make comparison difficult.

Podocarps generally occur on sites unfavourable for the vigorous development of broad-leaved species (Beveridge, 1983). Broad-leaved hardwoods require soils of moderate nutrient status, while podocarps are widely distributed on moderate to low fertility soils (Gibbs, 1983). Beech and kauri can tolerate low to very low nutrient levels, but the

advantage of the podocarps lies in their longevity and the persistence of their seedlings (Beveridge, 1983).

New soil surfaces favour the establishment of totara and kahikatea under a cover of small-leaved hardwood species (Beveridge, 1983). In Westland, thickets of Coprosma and, on dry, stony ground, totara, provide shelter for kahikatea seedlings (Wardle, 1974). As podsolization and gleying leads to decreased soil fertility, rimu gradually replaces kahikatea or, on better drained soils near the mountains, totara. The main requirement for podocarp regeneration is substantial breaks in an existing canopy, preferably created by the gradual disintegration of an overtopping tree (Beveridge, 1983).

Although these general observations have been made of the ecological behaviour of the podocarps, still little is known of their basic physiology or the genetic structure of their populations. These factors can have a marked influence on the distribution of the species, and the objective of this thesis is to examine some physiological and genetic factors which may affect the species' site preferences and their competitive ability in relationship to one another.

Due to their long life cycle and large geographic range, forest trees are subject to greater environmental diversity than most other plant species (Goddard and Hollis, 1984). Trees' evolutionary strategies for survival must include adaptations to temperature, nutrient availability, moisture and light. Variations in these parameters across geographic and climatic gradients may lead to adaptational differences between and within species as they compete for survival within the forest community.

Adaptation to growing season and winter temperatures must be among the strongest selective pressures acting on plant species. Nutrient availability appears to influence competition between species. For these reasons, and the fact that their effects are relatively easy to isolate, the two factors of nutrient supply and temperature were chosen as the

environmental variables on which to base a comparison of the physiology and genetic structure of rimu, kahikatea and totara under controlled conditions.

A knowledge of the genetic composition and structure of the species was also considered necessary for the interpretation of the results from the physiological work. Isozyme analysis was chosen as a tool to investigate the overall variability and the distribution of variation within and between populations of rimu, kahikatea, totara and some related species. To explore the genetic differences within the three species, an experiment testing provenance frost tolerance was carried out.

The purpose of this thesis, then, is to examine some of the major physiological and genetic factors which affect the distribution of rimu, kahikatea and totara, and which contribute to the dominance of rimu as a species in New Zealand.

CHAPTER II

TWO NUTRITION EXPERIMENTS

INTRODUCTION TO THE FIRST NUTRITION EXPERIMENT

There are major differences in adaptation to nutrient availability among and within tree species. Some species only grow in fertile areas, while others can tolerate low levels of fertility (Kozlowski, 1971). Plants that are adapted to low supplies of essential nutrients will be able to survive on nutrient-poor sites at the expense of species which lack such adaptations (Bannister, 1976). There also appears to be variability in response to nutrient availability within species; and Goddard and Hollis (1984) cite numerous examples of provenance differences.

Observations of the localized distribution of rimu, kahikatea and totara suggest that soil factors, including nutrient status, may determine the relative dominance of these species. Field observations indicate that kahikatea requires moist, nutrient-rich sites for its growth whereas rimu and totara have a more ubiquitous distribution (Cockayne, 1928; Cameron, 1960). On the West Coast of the South Island where rimu is widespread, plant succession closely parallels soil degradation (Sowden, 1986). As leaching removes nutrients from the soil, plant succession progresses from Olearia scrub to low kamahi and kahikatea forest to climax rimu forest within 3000 years. This progression may not be due to the change in nutrient status alone, as changing soil water and physical properties could also influence species distribution.

In potting soil in the glasshouse under conditions of equal fertility, totara and kahikatea appear to have a much greater growth rate than rimu (personal observation). This response could be due, among other things, to the larger seed

size and the early germination of these two species. On the other hand, it could be due to their superior ability to utilise available nutrients.

Rimu demonstrates many of the characteristics of plants of infertile soils (Grime, 1977). It has smaller leaves than totara, it appears to have a lower relative growth rate than kahikatea and totara; and it is less palatable than the other two species (Forest Research Institute, 1980). Rimu also has the most irregular seed production of all the podocarps (Beveridge, 1983), and this irregularity is another feature common in plants adapted to infertile sites (Chapin, 1980). An additional feature cited by Grime (1977) is the lack of a sharply defined seasonal pattern of growth. This appears characteristic of all three species however, and is more likely to be related to the equable climate of New Zealand, or to the species' evolutionary history (as discussed in Chapter IV).

Field observations of species' site preferences and associated soil nutrient requirements can be complicated by many interacting factors. The first nutrition experiment was therefore designed as a glasshouse trial, the purpose of which was to quantify and compare the responses of several provenances of rimu, kahikatea and totara to varying conditions of nutrient availability, while minimizing the impact of other environmental factors. It was hoped that an exploration of the species' differences in demand for and utilization of nutrients would contribute to the explanation of their distribution in relation to one another.

MATERIALS AND METHODS - FIRST NUTRITION EXPERIMENT

Seedling Origins

Seedlings for this trial were germinated in soil under constant light one month prior to the start of the experiment. Rimu seeds were germinated at alternating

temperatures of 30°/20°C with 12 hours at each temperature. Kahikatea and totara seeds were germinated at a constant temperature of 18°C. All seed had been kept moist at 4°C before germination.

Each species was represented by seedlings from two or three different locations. Rimu seed was from Waitutu, Jackson Bay and Hokitika. Kahikatea seed came from Christchurch, Charleston and Whirinaki Forest Park. The totara seed was collected from Invercargill and Raglan (see Appendix B for details of seed origin).

Nutrient Solutions

A primary reason for investigating the nutritional requirements of rimu, kahikatea and totara was that little is known of this subject. This lack of information, however, contributed to the difficulty of making appropriate nutrient solutions.

Soil surveys by the DSIR (1954; 1968) indicate that rimu and totara grow on sites ranging from low to high fertility, while kahikatea grows on sites of medium to high fertility. These surveys and Sowden's (1986) paper suggest that soils on which rimu is dominant have a total acid-extractable phosphorus content (as opposed to organic phosphorus) of 30 to 350 parts per million (ppm). Although not all of this phosphorus would be available for plant uptake, this was a basis from which the nutrient solutions were derived.

In work on Douglas-fir and Sitka spruce (Van den Driessche, 1968; 1973; 1978), the optimum level for nitrogen present in sand culture was a concentration of 50 ppm. The same level was found to be optimal for birch in solution culture (Ingestad, 1971). Quantities of 100 ppm of nitrogen maintained good growth in radiata pine seedlings in water and perlite cultures (Will, 1961), and this concentration was equivalent to levels in several New Zealand soils. For this

reason, 100 ppm was initially chosen as the maximum nitrogen concentration. The minimum nitrogen concentration was chosen to be 10 ppm and an intermediate concentration of 33 ppm was also included.

The proportions of the other elements in the nutrient mix were calculated from those in a MAF (1985) potting mix and Van den Driessche's (1978) solutions. The major elements were maintained at the same relative proportions in all mixes while calcium and the micro-nutrients were kept at a constant concentration. For the remainder of the discussion of this experiment, the various nutrient treatments will be referred to by their nitrogen concentration.

Eight weeks into the experiment, it was apparent that seedlings in the 100 ppm treatment were not reaching their maximum growth potential. Prior to this experiment, seedlings of all three species had performed very well in a MAF (1985) potting mix for trees and shrubs. Calculations revealed that nitrogen was present in this mix in concentrations of approximately 600 ppm, phosphorus at 400 ppm, and potassium at 500 ppm. For this reason, it was decided to increase the maximum nitrogen concentration to 600 ppm. Half of the pots in the 100 ppm treatment were separated and given an increased nutrient supply. To maintain an even distribution, half of the 33 ppm and 10 ppm treatment pots also had their nutrient supplies increased to 200 ppm and 60 ppm nitrogen respectively (Table 1).

On reflection, it would have been preferable to exceed the 600 ppm treatment and find the concentration of nutrients that provided a detrimental excess of supply. This would have been complicated however, by an uncertainty as to which element was at a toxic level and limiting growth, or whether the mix of the various nutrients was balanced.

Table 1: Total concentrations (ppm) of macro- and micro-nutrients added in one litre of solution to the six nutrient treatments over the course of the first nutrition experiment

Element	Source	Nutrient Treatments 600	200	100	60	33	10
MACRO-NUTRIENTS							
N	NH_4NO_3	600	200	100	60	33	10
P	KH_2PO4	180	60	30	18	10	3
K	KH_2PO_4+K_2SO_4	600	200	100	60	33	10
Ca	$CaCl_2$	150	150	150	150	150	150
Mg	$MgSO_4.7H_2O$	300	100	50	30	17	6
S		516	176	91	57	34	15
MICRO-NUTRIENTS							
Fe	Fe-EDTA	6	6	6	6	6	6
Mn	$MnSO_4.4H_2O$	0.2	0.2	0.2	0.2	0.2	0.2
Cu	$CuSO_4.5H_2O$	0.02	0.02	0.02	0.02	0.02	0.02
Zn	$ZnSO_4.7H_2O$	0.02	0.02	0.02	0.02	0.02	0.02
Mo	$NaMoO_4.2H_2O$	0.02	0.02	0.02	0.02	0.02	0.02
B	H_3BO_3	0.2	0.2	0.2	0.2	0.2	0.2

Experimental Design and Conditions

The experiment was carried out on a capillary bench in a heated glasshouse over 30 weeks of the summer and autumn. The bench was divided into six separate sections so that no mixing of the irrigation water could occur. In case of leakage, the original three nutrient treatments were placed in a sequence of increasing concentration down the bench.

There were two replicates of each nutrient treatment and each replicate consisted of six pots (16 cm X 12 cm), two for each species. Each pot contained three trees, chosen at random from two provenances and planted in sterile, non-toxic vermiculite. Thus there totalled six trees of each species in each replicate of each nutrient treatment (Plate 1).

Plate 1: The layout of the first nutrition experiment

The arrangement of pots and replicates within nutrient treatments was re-randomized bi-weekly; however the arrangement of nutrient treatments could not be randomized due to the constraints of the capillary watering system.

A capillary watering system was chosen to minimize leaching of nutrients from the pots. At three week intervals, the benches were dried for a day, and then the nutrient solutions were added. After another day, water was re-introduced to the bench, and the evaporative gradient through the pots was intended to prevent nutrient loss. This system did not work perfectly however, as significant algal bloom in the 200 ppm and 600 ppm treatment sections indicated some loss of nutrients.

The seedlings were only one to two centimetres tall at the beginning of the experiment, thus the nutrient solutions were added in increasing concentrations to maximize uptake. The levels of the various elements depicted in Table 1 are the total concentrations of nutrients added in one litre of solution to each pot in a nutrient treatment over a 19 week period. For the 10 ppm, 33 ppm and 100 ppm treatments, one

twentieth of each nutrient solution was added in the first week, two twentieths in the fourth week, three twentieths in each of the seventh, tenth and thirteenth weeks, and four twentieths in the sixteenth and nineteenth weeks. The 60 ppm, 200 ppm and 600 ppm treatments received the same nutrient solutions as the 10 ppm, 33 ppm and 100 ppm treatments respectively for the first four applications; and then their solution concentrations were increased to reach the final, stated levels. This was intended to supply the increasing demands of the growing plants with minimum nutrient loss.

The glasshouse temperature was thermostatically controlled, but there were large fluctuations over the duration of the experiment. The glasshouse averaged 22°C (range 18° to 37°C) during the 30 week period. The temperature was set for a minimum of 20°C, so the times the temperature was below this level were rare.

Light levels varied naturally with the time of day and season. The maximum light intensity in the glasshouse at mid-afternoon on a clear day was about 1000 $\mu molm^{-2}s^{-1}$.

Relative humidity also varied with time of day and season, and averaged 60 percent. The range was from 20 to 90 percent over the 30 week period.

Measurements of Growth and Photosynthesis

The height of each seedling was measured at the beginning of the experiment and again every month for the duration. In this and all other experiments, the height of totara was measured from the cotyledons to the tip of the terminal bud. For rimu and kahikatea, height was measured from the cotyledons to the tips of the leaves highest above the soil. At the end of the trial, seedlings were carefully removed from the vermiculite, measured for height, number of branches and stem diameter at ground level. The trees were then divided into root, stem and leaf portions, oven-dried at

80°C for 48 hours and weighed.

One day prior to the seedlings' harvest, some were measured for photosynthetic rate. Four randomly selected seedlings of each species in the 10 ppm, 200 ppm and 600 ppm treatments were measured using the LI-6000 Portable Photosynthesis System (LI-COR Inc. Lincoln, Nebraska, U.S.A.) incorporating a 1 l cuvette (Plate 2). Two measurements were taken per tree, and the length of measurement depended on the time required to obtain a 30 ppm drawdown in CO_2 concentration. These readings were taken on the same day in as uniform conditions as possible (cuvette temperature: 22° to 27°C, relative humidity: 32 to 45 percent, light intensity: 250 to 330 $\mu molm^{-2}s^{-1}$). All photosynthetic measurements were expressed on a total foliage area basis. Leaf area was measured with a Delta-T Area Meter.

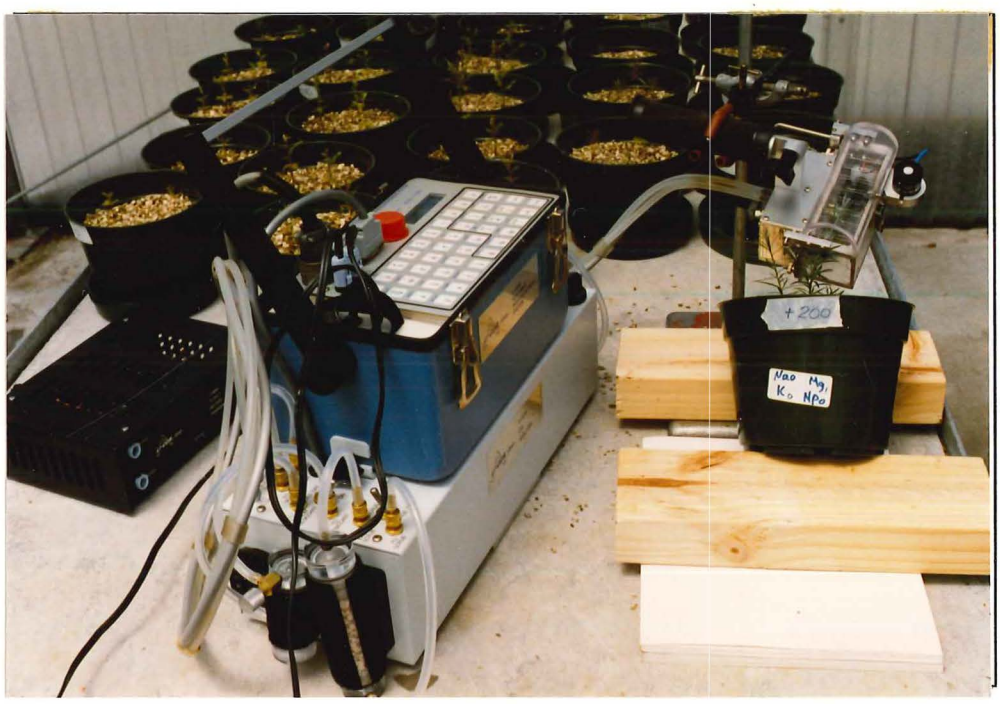

Plate 2: Measurement of net photosynthetic rate using the LI-6000 Portable Photosynthesis System

Tissue Analysis

Once the seedlings were dried and individually weighed, the dry matter for all seedlings of each species in each nutrient treatment was bulked. The exception to this was one pot of totara in each of the 10 ppm and 60 ppm treatments. The trees in these two pots grew at a strikingly higher rate than the remaining trees in those treatments, therefore their dry matter was bulked for separate analysis.

The bulked seedlings were ground to a fine powder, digested, and analyzed for concentrations of nitrogen, phosphorus, potassium, calcium and magnesium (Nicholson, 1984). Measurements of the nitrogen and phosphorus concentrations were made with a Pye-Unicam Ac1 auto-analyzer coupled with a Phillips SP6 visible light spectrophotometer. The same measurements for potassium, calcium and magnesium were taken with a Varian Techtron 1100 atomic absorption spectrophotometer. This work was conducted in the laboratory of the Forest Research Centre, Christchurch.

Analysis

(i) Growth. The monthly height measurements were used to produce growth curves of the natural log of height versus time. An analysis of variance for each species was carried out for all measured growth variables (i.e. total weight, root weight, stem weight, leaf weight, height, number of branches and stem diameter) and also the ratio of root and leaf weights to total weight. Provenances were identified separately. A split plot model was used for the analysis as follows (assuming two provenances) (see Appendix C for sums of squares):

	SOURCE OF VARIATION	df
Whole Plot	Nutrient Treatment	5
	Replicate	1
	Error A	5
Split Plot	Provenance	1
	Treatment X Provenance	5
	Error B	54
	Total	71

The 'Error B' term was comprised of the following elements:

SOURCE OF VARIATION	df
Replicate X Provenance	1
Treatment X Replicate X Provenance	5
Between Tree	2
Treatment X Tree	10
Replicate X Tree	2
Provenance X Tree	2
Treatment X Tree X Replicate	10
Treatment X Tree X Provenance	10
Replicate X Provenance X Tree	2
Treatment X Replicate X Provenance X Tree	10

As the variation explained by the elements comprising the error term was insignificant, these terms were pooled to increase precision.

Means for each variable, species and nutrient treatment were compared by Scheffe's test with a confidence level of 0.95. The linear and quadratic components of the analyses of variance were also calculated and tested for significance (Appendix D).

The unusually large totara seedlings from the 10 and 60 ppm treatments, when measured for nutrient concentration, were found to have much higher nutrient concentrations than the other totara seedlings in those treatments (Appendix E). For this reason it was deemed justifiable to exclude these trees from the analysis. This produced an unbalanced design, and the analyses of variance were done using the General Linear Model Procedure of SAS (1985).

(ii) Photosynthesis. The two measurements of photosynthetic rate for each tree were meaned, and then the net photosynthetic rates of all species in all three nutrient treatments were compared by analysis of variance. In this case the model was as follows (see Appendix F for sums of squares):

SOURCE OF VARIATION	df
Nutrient Treatment	2
Species	2
Treatment X Species	4
Error	27
Total	35

The 'Error' term was comprised of the following elements:

SOURCE OF VARIATION	df
Between Trees	3
Treatment X Trees	6
Species X Trees	6
Treatment X Species X Trees	12

As the variation explained by these interactions was not significant, they were pooled to increase precision. The means were compared by Scheffe's test with a confidence level of 0.95.

(iii) Relative Growth Rate. To enable calculation of the relative growth rate of each species in each nutrient treatment, regressions were calculated, by species, for the natural log of total weight against the natural log of height. Correlations were good (R^2=0.6 for rimu, 0.8 for kahikatea and 0.9 for totara, with 72 observations for each species) and from these regression equations, the starting weight of each of the species in each nutrient treatment was calculated.

Relative growth rate in each nutrient treatment was calculated as:

$$\frac{\ln(\text{total weight at time}_t) - \ln(\text{total weight at time}_0)}{\text{time}_t - \text{time}_0}$$

and these values were plotted.

(iv) Tissue Analysis. The measured concentrations of nitrogen, phosphorus and potassium in the seedling tissues were compared using an analysis of variance. The model, as follows, was similar to the comparison of photosynthetic rates (see Appendix G for sums of squares).

SOURCE OF VARIATION	df
Nutrient Treatment	5
Species	2
Treatment X Species	10
Error	18
Total	35

The 'Error' term was comprised of the following elements:

SOURCE OF VARIATION	df
Between Samples	1
Treatment X Samples	5
Species X Samples	2
Treatment X Species X Samples	10

As the variation explained by these interactions was not significant, they were pooled to increase precision. The means were again compared by Scheffe's test with a confidence level of 0.95.

The measures of nutrient concentration in each nutrient treatment for each species were then converted to an 'uptake' basis by multiplying the entire tree concentrations by total weight and then multiplying this number by three (three trees per pot). This resulted in a figure for the weight of an element in the trees in one pot which was divided by the weight of element added to the pot. Multiplied by 100, this value represented the percentage uptake of nutrients in each pot. This was then divided by the average root weight of trees in that nutrient treatment, resulting in the tabulated figures of the percentage uptake of nutrients per unit of root weight, called the 'uptake efficiency index'.

RESULTS OF THE FIRST NUTRITION EXPERIMENT

Growth

For all three species studied, nutrition had a significant effect on growth. Table 2 presents measured growth variables for the three species and six nutrient treatments. For all species and all variables, increasing nutrition significantly increased growth ($p=0.01$). In terms of total weight and height, the three species differed significantly ($p=0.0001$) from one another. Totara had the heaviest seedlings, followed by kahikatea and then rimu; however, the kahikatea seedlings were the tallest, followed by totara and then rimu (Plate 3).

Table 2: Tests of significance of measured variables in the first nutrition experiment [1]

Rimu Nutrient Treatment	Mean	Kahikatea Nutrient Treatment	Mean	Totara Nutrient Treatment	Mean
TOTAL WEIGHT (g)					
600	0.158	600	0.548	600	1.062
200	0.109	200	0.307	100	0.505
100	0.101	100	0.270	200	0.376
60	0.092	33	0.184	33	0.265
33	0.087	60	0.161	60	0.242
10	0.078	10	0.123	10	0.159
LEAF WEIGHT (g)					
600	0.080	600	0.234	600	0.549
200	0.051	200	0.118	100	0.246
100	0.044	100	0.092	200	0.191
60	0.040	33	0.056	60	0.108
33	0.034	60	0.052	33	0.107
10	0.031	10	0.038	10	0.060
ROOT WEIGHT (g)					
600	0.046	600	0.164	600	0.223
100	0.040	200	0.118	100	0.166
200	0.039	100	0.118	200	0.119
60	0.038	33	0.094	33	0.117
33	0.038	60	0.077	60	0.099
10	0.035	10	0.064	10	0.078
HEIGHT (mm)					
600	77.6	600	184.0	600	172.2
200	58.3	200	102.7	100	75.9
100	47.8	100	90.5	200	72.2
60	46.0	60	69.6	60	39.7
33	41.8	33	64.3	33	35.9
10	41.3	10	51.0	10	23.7
DIAMETER (mm)					
600	1.01	600	1.99	600	1.99
33	0.92	200	1.55	100	1.55
60	0.92	100	1.45	200	1.39
200	0.90	33	1.28	33	1.25
100	0.90	60	1.22	60	1.18
10	0.81	10	1.11	10	1.06

[1] Values spanned by the same bar are not significantly different (p=0.05).

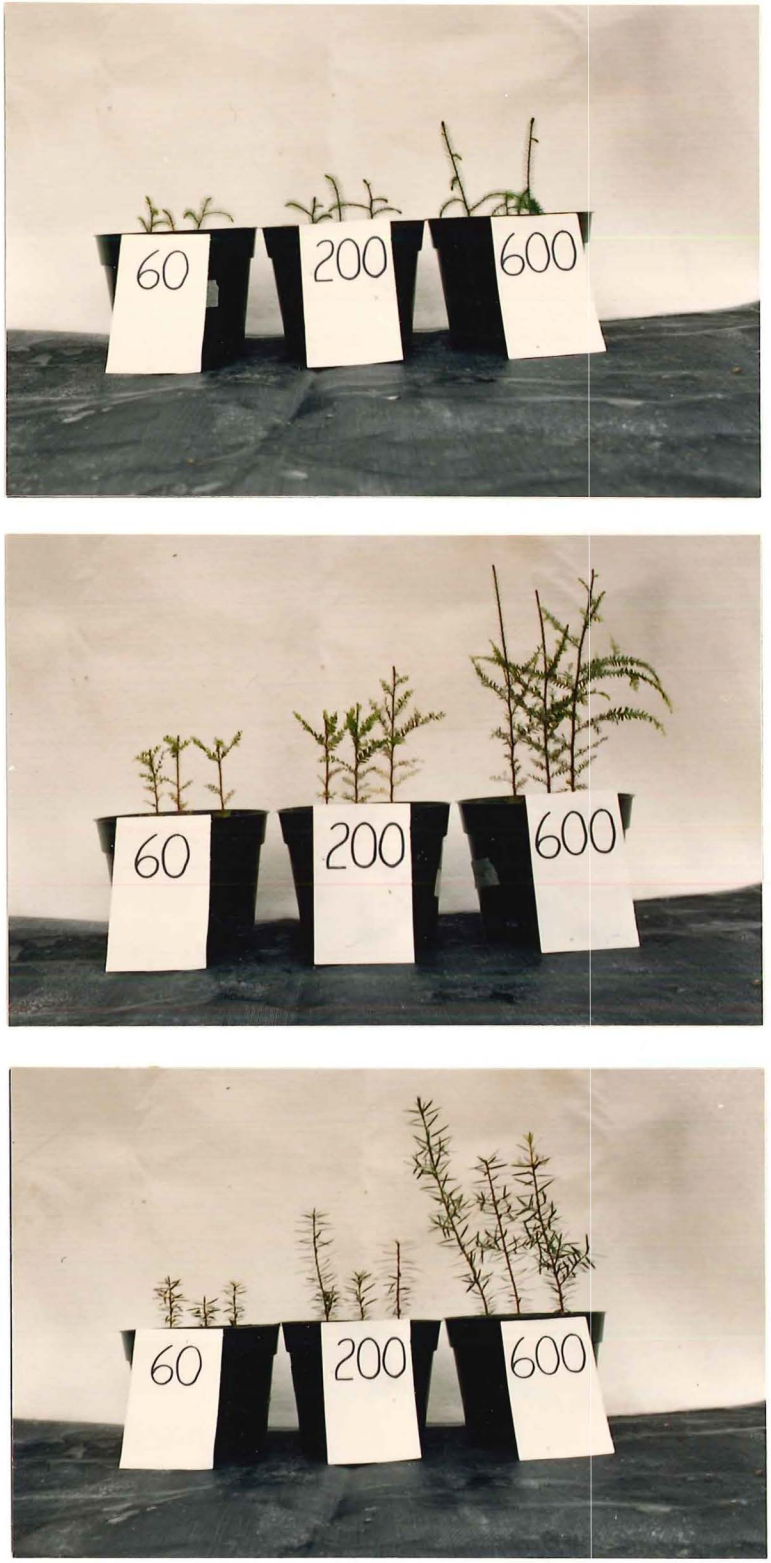

Plate 3: Average seedlings of rimu (top), kahikatea (centre) and totara (bottom) from three nutrient treatments

Even with the anomalous seedlings in the 10 ppm and 60 ppm nutrient treatments, totara showed significant differences in growth between treatments. If the anomalous results were removed from the calculations, the significance became much greater.

As well as differences between species, there were significant differences in growth within species (Appendix C). For rimu, the Jackson Bay provenance had significantly ($p=0.05$) greater growth than the Waitutu or Hokitika provenances. The kahikatea seedlings from Charleston grew better than those from the Central North Island or Christchurch; however, there were no significant differences in growth between totara provenances.

While growth of all species was significantly affected by nutrition, the rimu seedlings did not seem to be as responsive to changing nutrition as the seedlings of totara and kahikatea. The Scheffe tests for rimu did not separate between nutritional treatments as clearly as for kahikatea and totara, and the slopes for the linear components of the analyses of variance were consistently lower for rimu than the other two species (Appendix D).

The response in growth to increased nutrition was linear for all species, and the addition of a quadratic component did not account for a significant amount of variation. For the linear regressions of growth and nutrient treatment, totara had the steepest slopes of the three species, however kahikatea produced the best correlations.

Figures 1, 2 and 3 show the natural logarithm of height growth for the three species with time, and in all cases a distinct separation by nutritional treatments can be seen, despite initial differences in seedling size.

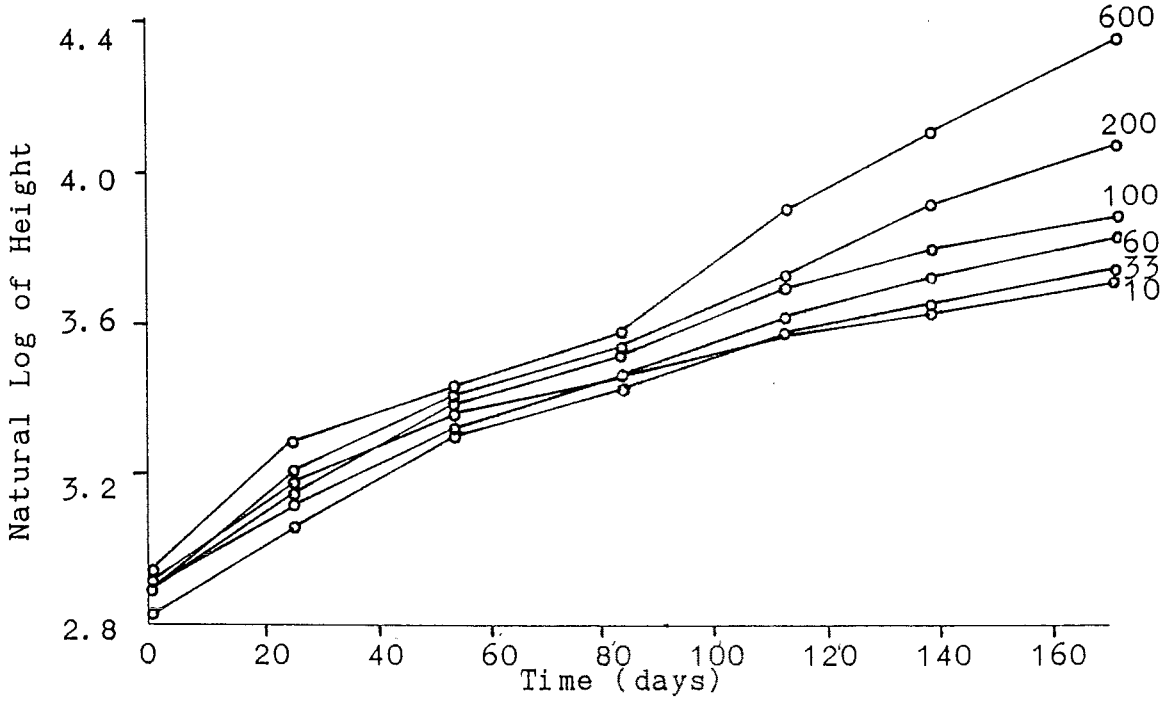

Figure 1: Plot of the natural log of height of rimu seedlings versus time for six nutrient treatments

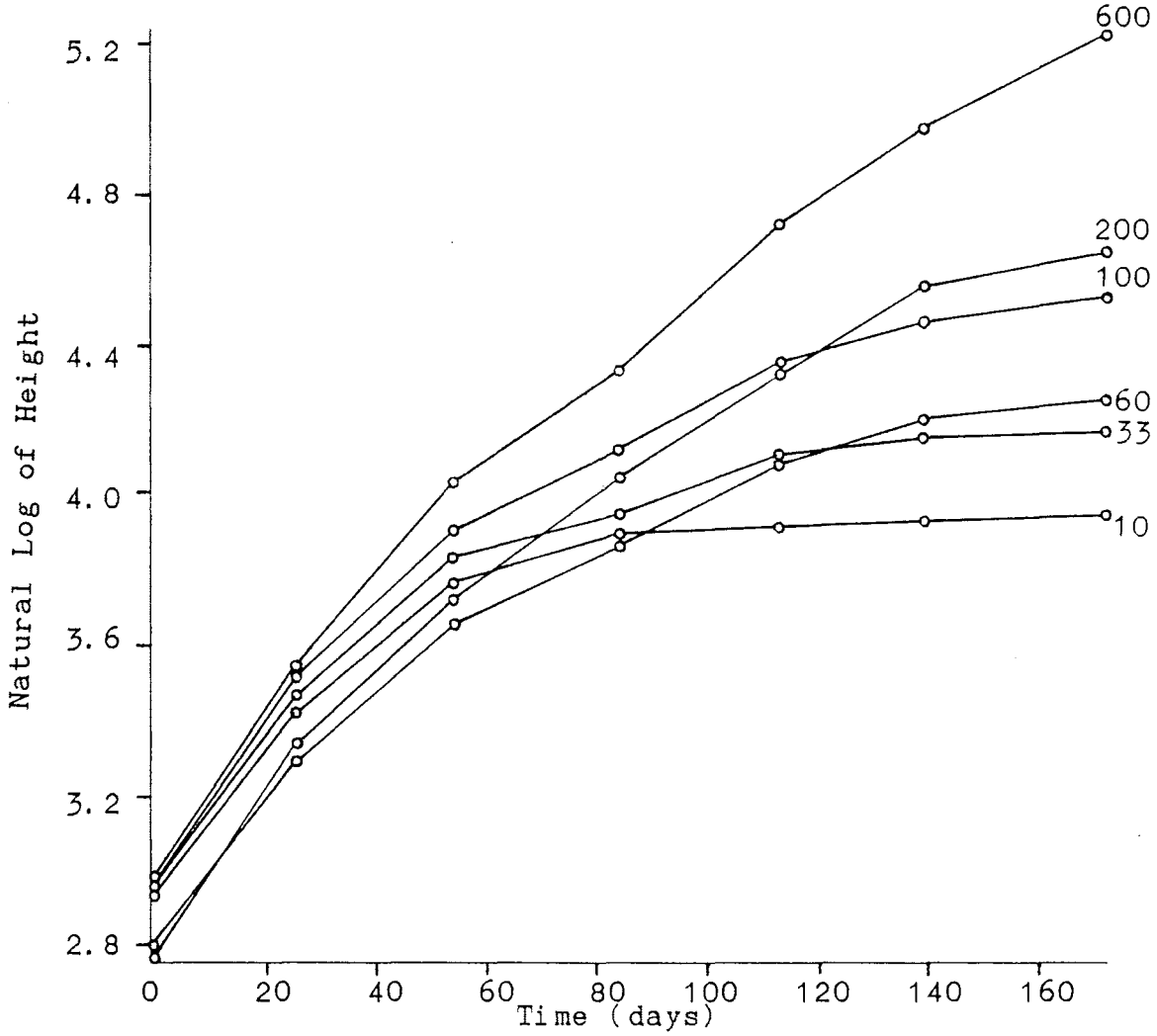

Figure 2: Plot of the natural log of height of kahikatea seedlings versus time for six nutrient treatments

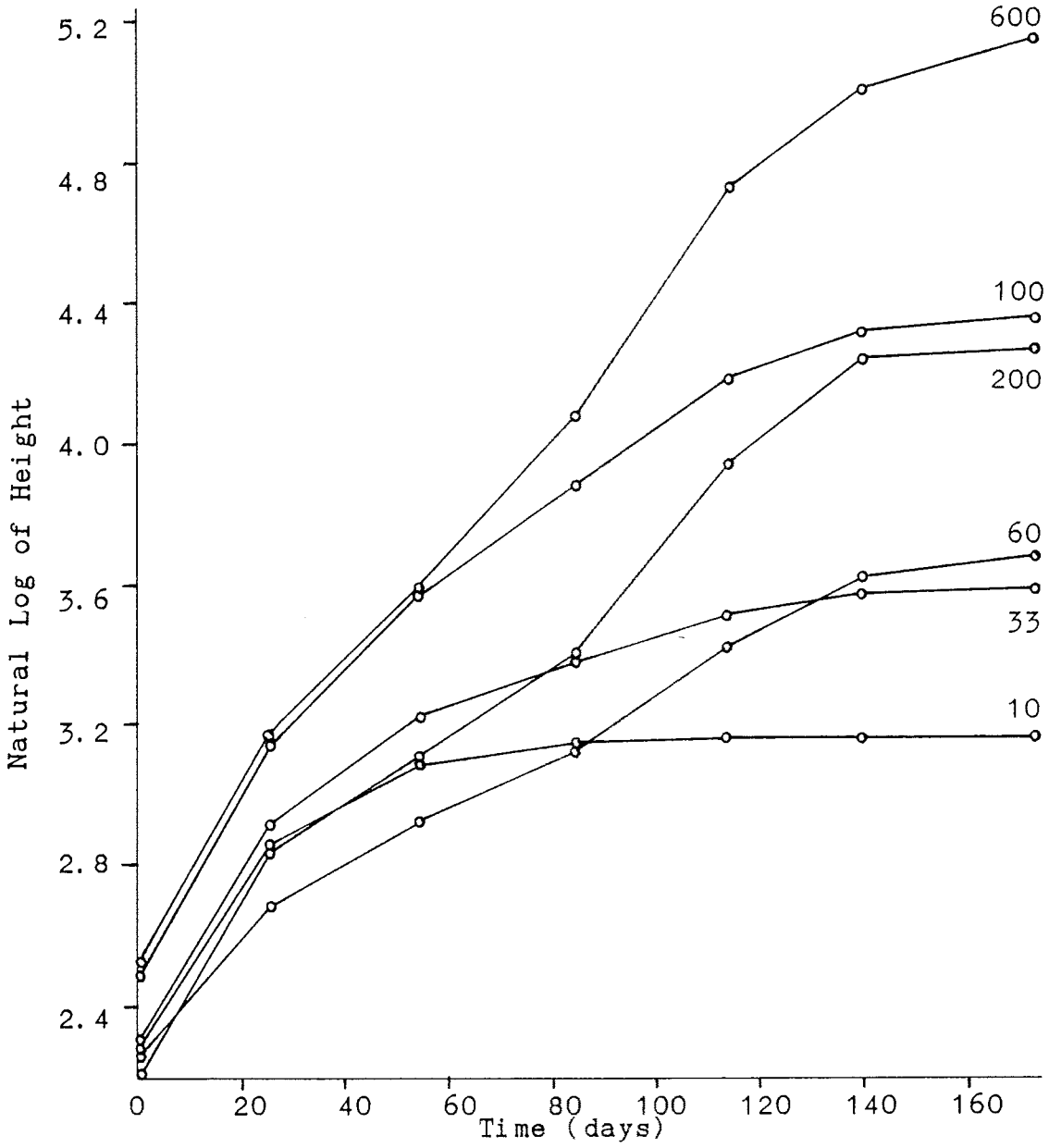

Figure 3: Plot of the natural log of height of totara seedlings versus time for six nutrient treatments

Relative Growth Rate

The effect of initial seedling size was removed in the graphs of relative growth rate (Figure 4). Here, growth increases uniformly with the level of nutrition in all species.

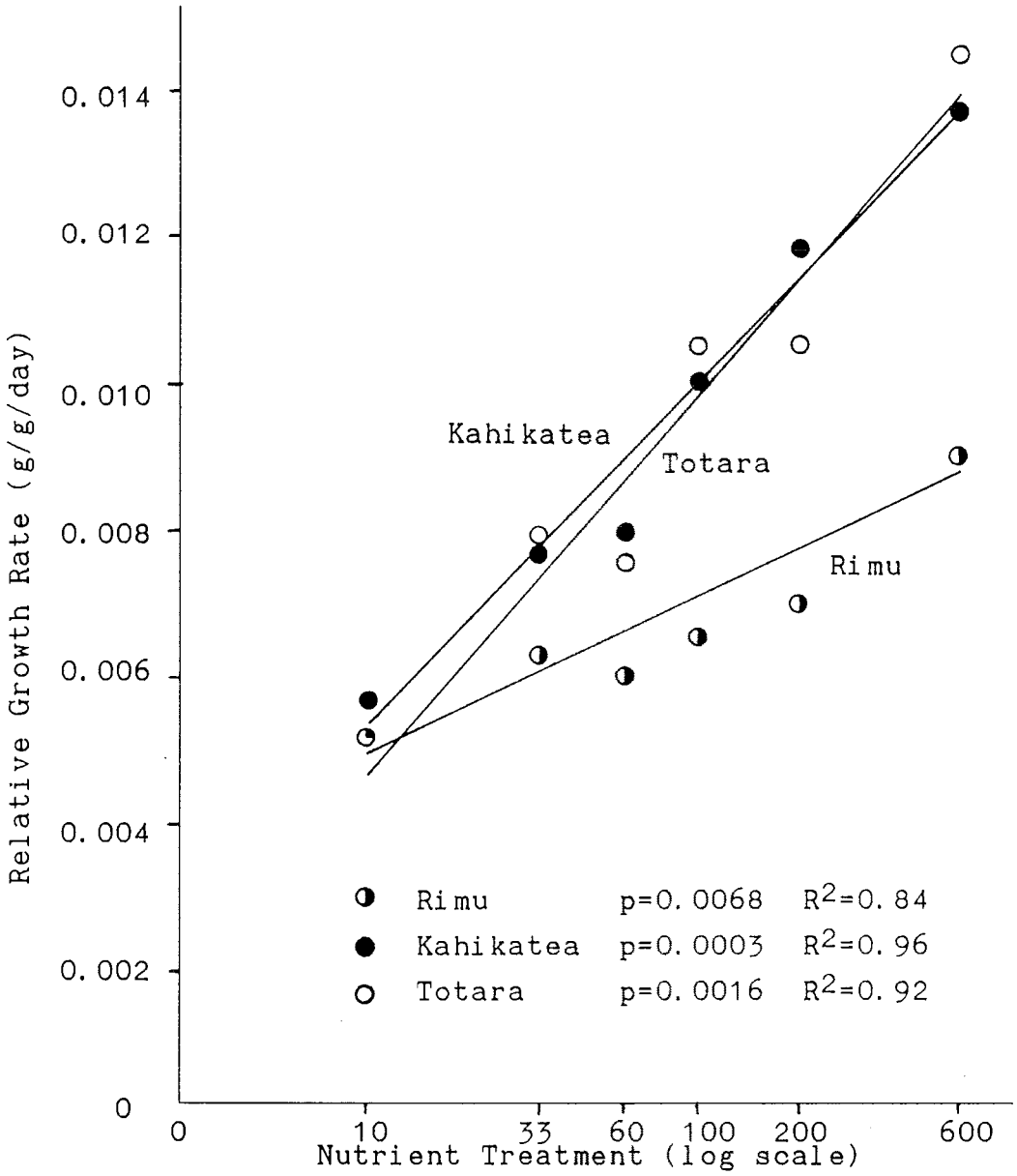

Figure 4: Plot of relative growth rate for three species by nutrient treatment (estimated starting weights)

The graph of relative growth rate shows that totara and kahikatea had a much more rapid response to increased nutrition than rimu. In the 10 ppm treatment, the relative growth rates of the three species were equal; but between the 10 ppm and 200 ppm treatments, the relative growth rate of totara and kahikatea almost doubled, while rimu's relative growth rate increased by less than half.

Allocation of Resources

For all species, the ratio of leaf weight to total weight was significantly affected by nutrient treatment, with a greater allocation of dry matter to the leaves at higher nutrient treatments (Table 3). The three species had significantly different leaf weight ratios (p=0.0001) with totara allocating the most resources to leaf growth, particularly at higher levels of nutrition. Rimu had the second highest leaf weight ratio and kahikatea the third.

Table 3: Test of significance of leaf weights and root weights as a proportion of total weight in the first nutrition experiment [1]

Rimu Nutrient Treatment	Mean	Kahikatea Nutrient Treatment	Mean	Totara Nutrient Treatment	Mean
LEAF WEIGHT/TOTAL WEIGHT					
600	0.502	600	0.422	600	0.517
200	0.463	200	0.381	200	0.504
100	0.439	100	0.334	100	0.486
60	0.434	60	0.317	60	0.441
10	0.399	10	0.307	33	0.394
33	0.395	33	0.306	10	0.380
ROOT WEIGHT/TOTAL WEIGHT					
10	0.455	10	0.516	10	0.492
33	0.440	33	0.506	33	0.467
60	0.412	60	0.481	60	0.413
100	0.393	100	0.444	100	0.331
200	0.363	200	0.384	200	0.323
600	0.302	600	0.303	600	0.212

[1] Values spanned by the same bar are not significantly different (p=0.05).

Taking root weight as a proportion of total weight (Table 3), it can be seen for all species that, at lower levels of nutrition, there was a significantly greater allocation of dry matter to the roots. The difference between species in root weight ratio was also significant

(p=0.0001), and kahikatea put the most resources into root production.

Totara showed the greatest change in resource allocation with improving nutrition, switching the greatest proportion of resources from roots to leaves. Rimu demonstrated the least response in resource allocation (Plate 4).

Photosynthesis

Increased growth of the three species at higher nutrient levels was a result of increased photosynthetic rate and increased leaf area.

The photosynthetic rates of the different species were not strictly comparable due to varying leaf morphology and stomatal arrangement. There was however, a significant difference (p=0.01) in the photosynthetic rate between nutrient treatments within each species (Table 4). This shows quite clearly that the photosynthetic rates of these species were related to their level of nutrition.

Table 4: Mean photosynthetic rates by species in the first nutrition experiment [1]

Rimu		Kahikatea		Totara	
Nutrient Treatment	Photosynthesis ($mgCO_2/m^2s$)	Nutrient Treatment	Photosynthesis ($mgCO_2/m^2s$)	Nutrient Treatment	Photosynthesis ($mgCO^2/m^2s$)
600	0.124	200	0.114	600	0.128
200	0.124	600	0.108	200	0.087
10	0.071	10	0.043	10	0.036

[1] Values spanned by the same bar are not significantly different (p=0.05).

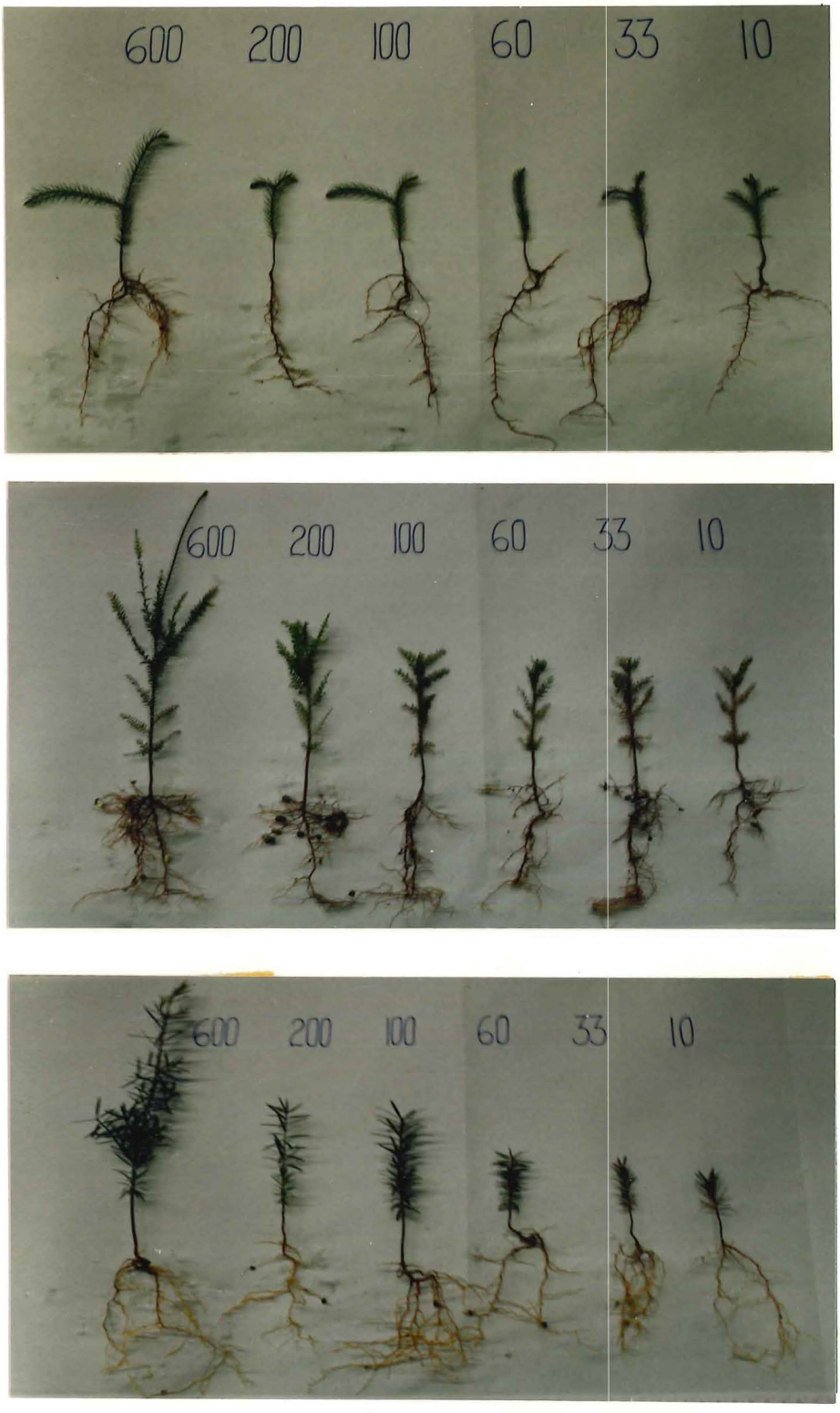

Plate 4: Average whole trees of rimu (top), kahikatea (centre) and totara (bottom) from six nutrient treatments

Tissue Analysis and Nutrient Uptake

Nutrient treatment significantly ($p=0.0001$) affected the concentrations of all elements analyzed in the seedling tissues. Higher nutrient treatments resulted in higher tissue concentrations.

Concentrations of two of the macro-nutrients, N and P, in seedlings of the species generally showed rimu to have the greatest uptake per unit weight (Table 5). The small size of the rimu seedlings, however, meant that the actual number of grams of element per seedling was lower than for the other species. Totara appeared to have a greater uptake of K than did the other species. Within-plant concentrations of N, P and K changed significantly with the amounts added, but concentrations of Mg did not. The elements were accumulated by the seedlings to a point where they were much more concentrated than in the original solutions.

The table of uptake efficiency indices (Table 6) shows that rimu generally was more efficient in the uptake of nutrients than the other two species. The difference was greatest in the lowest nutrient treatments. In the 600 ppm treatment, for all elements but P, totara had the highest uptake per unit root weight, although this did not apply to uptake per unit plant weight (Table 5). This species' high demand for K and Ca can again be seen.

While for most elements uptake decreased as nutrient supply increased, the absorption of Ca, which was applied at a constant rate, increased with increasing nutrient supply.

It should be noted that while the overall concentration of elements within the plants increased with increasing nutrient level, the percent uptake of the elements per unit root weight decreased. This is a result of the root's apparent increased efficiency of absorption at lower nutrient levels, and the general decrease of root weight with decreased nutrient availability. Thus, while the uptake, as a percentage of what was added, increased at low nutrient

levels, the total root weight was lower. This resulted in an even larger percentage uptake per unit root weight.

Table 5: Concentrations of nitrogen, phosphorus and potassium in whole seedlings in the first nutrition experiment [1]

Rimu		Kahikatea		Totara	
Nutrient Treatment	Mean (%)	Nutrient Treatment	Mean (%)	Nutrient Treatment	Mean (%)
NITROGEN					
600	1.41	600	1.53	600	1.10
200	1.22	200	1.18	200	0.90
100	1.09	100	1.08	100	0.79
60	1.02	60	1.01	60	0.74
10	0.92	10	0.81	33	0.63
33	0.88	33	0.80	10	0.55
PHOSPHORUS					
600	0.33	600	0.15	600	0.15
200	0.21	200	0.12	200	0.11
100	0.16	60	0.10	60	0.09
60	0.12	100	0.09	100	0.08
33	0.09	33	0.07	33	0.06
10	0.08	10	0.06	10	0.04
POTASSIUM					
600	1.36	600	1.15	600	1.41
200	0.97	200	0.94	200	1.26
100	0.86	100	0.85	60	1.11
60	0.74	60	0.82	100	1.10
10	0.74	10	0.78	33	1.08
33	0.68	33	0.77	10	0.96

[1] Values spanned by the same bar are not significantly different (p=0.05).

Table 6: Uptake efficiency indices in the first nutrition experiment [1]

Rimu Nutrient Treatment	Rimu Mean (%/g)	Kahikatea Nutrient Treatment	Kahikatea Mean (%/g)	Totara Nutrient Treatment	Totara Mean (%/g)
NITROGEN					
600	23.9	600	19.5	600	26.5
200	50.0	200	45.8	200	42.9
100	84.6	100	74.6	100	72.3
60	123.7	60	106.5	60	89.9
33	181.6	33	141.5	33	129.1
10	617.1	10	464.1	10	338.5
PHOSPHORUS					
600	18.7	600	8.5	600	12.0
200	28.2	200	15.2	200	16.9
100	40.0	100	20.3	100	25.3
60	47.4	60	33.8	60	35.3
33	60.5	33	41.5	33	38.5
10	162.9	10	125.0	10	91.0
POTASSIUM					
600	13.0	600	11.0	600	18.8
200	22.8	200	20.3	200	33.6
100	37.5	100	32.2	100	56.6
60	50.0	60	48.0	60	76.8
33	78.9	33	77.6	33	125.6
10	277.1	10	253.1	10	328.2
CALCIUM[2]					
600	54.3	600	50.6	600	79.8
200	58.9	200	38.1	200	63.0
100	52.5	100	39.8	100	64.5
60	50.0	60	36.4	60	47.5
33	47.4	33	31.9	33	49.1
10	45.7	10	34.4	10	41.0
MAGNESIUM					
600	9.8	600	7.3	600	11.7
200	24.6	200	16.9	200	23.5
100	44.0	100	33.5	100	43.4
60	72.4	60	55.2	60	56.6
33	115.8	33	63.8	33	96.6
10	340.0	10	195.0	10	241.0

[1] Uptake efficiency index = $\frac{\left[\frac{\text{\# g element in trees in pot}}{\text{\# g element added to pot}}\right] X100}{\text{root weight}}$

[2] Ca was supplied at a constant concentration in all treatments.

DISCUSSION OF THE FIRST NUTRITION EXPERIMENT

Effect of Nitrogen

In the experiment under discussion, there were significant differences in response to nutrient availability both between and within species. The differences between nutrient treatments consisted of differences in the concentrations of all macro-nutrients, but of these, nitrogen was likely to have been very important. Meristematic tissues producing vegetative growth have an active protein metabolism; thus during the vegetative stage, nitrogen nutrition controls the growth rate of a plant to a large extent (Mengel and Kirkby, 1979).

Generally, nutritional experiments with forest trees do not exceed a 200 ppm nitrogen supply (Hewitt, 1966), and research indicates that forest trees maintain good growth with a 50 to 100 ppm nitrogen supply (Ingestad, 1971; Van den Driessche, 1968; 1973; 1978; Will, 1961). In many experiments, the objectives are to extrapolate optimum levels of supply and critical foliage levels to field situations, and thus the basic nutrient concentrations are similar to those in the soil (Will, 1961). In the experiment under discussion, the objective was to compare the response of the three species over a large range of conditions, thus nutrient supplies were provided far in excess of normal field conditions. It is possible that the rimu, kahikatea and totara seedlings were not actually exposed to the amount of elements added, as the growth of algae indicated some nutrient loss from the pots; thus specific comparison of these results with other trials should be made cautiously.

Species and Provenance Response

The growth of all three species in this trial increased with increased nutrient supply. This response was linear. There is evidence that the high nutrient requirements of conifer seedlings may continue to an age of 20 to 30 years (Walker et al., 1955); thus the results of this experiment could well be applicable to saplings of the three species.

The response of increased growth with increased nutrient supply extended to all provenances of the three species, but some provenances had greater overall growth than others. The West Coast provenances of rimu and kahikatea grew larger than the seedlings of the other provenances; but it is not known whether this was due to earlier germination, larger seedling size or a superior ability to utilize available nutrients. As there was not enough dry matter to do separate tissue nutrient analyses for the different provenances, this question cannot be answered.

While there were no significant differences in growth between the provenances of totara, one pot in each of the 10 ppm and 60 ppm treatments contained abnormally large seedlings (Plate 5). Baylis et al. (1963) demonstrated that two year old totara seedlings infected by mycorrhizal fungi had mean dry weights approximately seven times greater than those of non-infected seedlings. It is possible that the anomalous totara seedlings in the two pots might have been infected by mycorrhizal fungi, and this could have accounted for their doubling in size and increased tissue nutrient concentrations compared to the other seedlings.

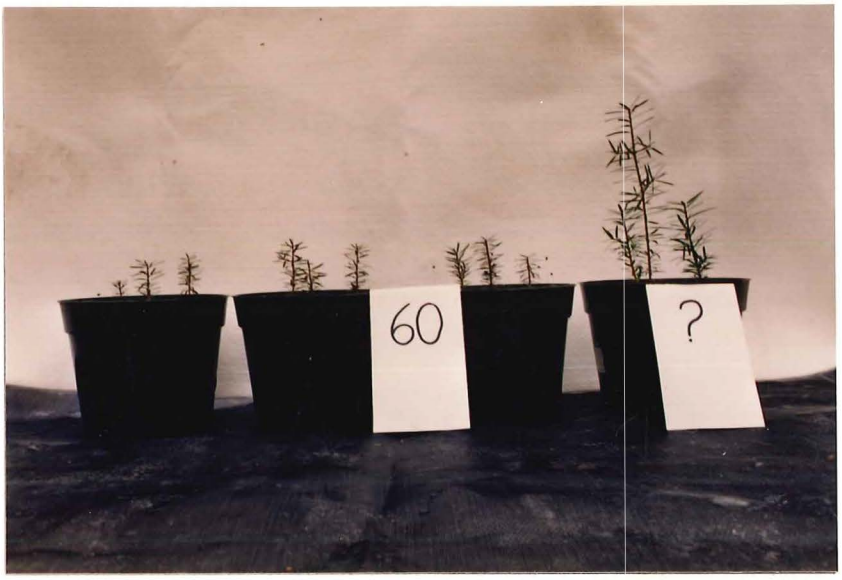

Plate 5: Three pots of normal totara seedlings and one pot of anomalous seedlings from the 60 ppm nutrient treatment

Allocation of Resources

The allocation of resources within the seedlings changed with nutrient supply. At lower levels of nutrition, there was a greater apportionment of growth to roots and less to leaves than at higher levels of supply. This is a common response in plants, and it is hypothesized that because root meristems are close to the nutrient supply, they receive an extra share of nutrients and therefore grow more rapidly than shoot meristems (Chapin, 1980). It is also a response that would be expected as, at lower levels of supply, the plant would need a larger root surface area to obtain its minimum nutritional requirements, and the amount of foliage that could be supported per unit of root would be reduced.

Totara and rimu had a similar apportionment of photosynthate to leaves; however kahikatea had a much lower leaf weight ratio. This species seems adapted to allocate a large percentage of resources to roots, particularly in conditions of low fertility. Totara and kahikatea exhibited a fairly high plasticity in their apportionment of resources

to roots. Rimu, on the other hand, did not show quite as much variation. This agrees with Grime's (1977) observation that species characteristic of favourable habitats show greater flexibility in allocation pattern than do those from stressful environments.

Photosynthetic Response

Increased nutrition increased the photosynthetic rates of all species as well as increasing the allocation of resources to leaf production. All elements essential for plant growth have been reported to affect rates of photosynthesis in higher plants. Nitrogen, magnesium and phosphorus are directly involved in the basic photosynthetic processes, and other elements indirectly affect photosynthesis or the utilization of photosynthate (Linder and Rook, 1984). Thus, it has been well documented that photosynthetic rate is proportional to nutrient supply, especially nitrogen supply, over a broad range (Chapin, 1980).

Response of Relative Growth Rate

The higher photosynthetic rates over larger leaf areas with increased nutrient supplies naturally led to increased relative growth rates (Figure 4). As the photosynthetic rates of the three species cannot be directly compared due to differences in leaf morphology and stomatal arrangement, it cannot be concluded that rimu's comparatively large leaf area ratio and photosynthetic rate should produce a high relative growth rate. In fact, the opposite trend is evident as rimu had the lowest relative growth rate of the three species in the trial.

The only point at which the relative growth rates of the three species were equal was at the very lowest nutrient treatment. From this point, the growth rates of kahikatea and totara increased rapidly compared to rimu.

Chapin (1980) documents evidence of species from infertile sites showing less growth response to increased nutrient availability than species from fertile sites. He suggests several possible advantages of an inherently low growth rate in an infertile habitat:

1) Slowly growing species with low assimilation rates are less likely to exhaust limited soil nutrient reserves.

2) On an infertile site, a species with an inherently low growth rate may be functioning closer to its optimal growth and metabolic rate, and therefore may be more fit than a species experiencing a large reduction in growth rate under those conditions.

3) Slowly growing species may experience luxury consumption during nutrient flushes and will be able to survive longer on these reserves than quickly growing species.

4) Meristems of slowly growing species maintain activity and the potential to respond to improved conditions, whereas meristems of quickly growing species may become dormant when nutrient reserves are exhausted and thus will no longer be able to respond.

Tissue Analyses and Uptake Response

Tissue from entire trees had to be analyzed for nutrient concentration in this experiment due to a lack of dry matter. This produced measurements of tissue nutrient concentration lower than the foliar concentrations commonly measured in other experiments. In spite of this, the levels of N, P, K, Mg and Ca in the 100 ppm, 200 ppm and 600 ppm regimes were generally above the marginal limits for foliar nutrient concentrations of several other coniferous species (Mead, 1984).

Although species from infertile habitats may show little growth response to improved nutrition, they often increase the concentration of elements in their tissues (Chapin, 1980). In general, this appears to be true for

rimu, as at higher rates of nutrition this species had greater element concentrations in its tissues than the other two species. Rimu seemed to be particularly able to absorb phosphorus as it had much higher concentrations of this element in all treatments than did the other two species. The exception to rimu tissue's higher element concentrations was for potassium as totara appeared to have a very high demand for this element.

All three species had the greatest sensitivity of response to changing phosphorus levels. The concentrations of this element in the seedling tissues showed the greatest range of all those analyzed; thus it appears that the demand for this element is strong, and the seedlings are adapted to take advantage of increased supplies. J.C. van Dorsser (pers. comm.) states that the maximum growth response of rimu seedlings can be obtained with a combination of phosphorus, nitrogen and potassium fertilizers, and it can be seen that all seedlings in this experiment were sensitive to concentrations of these elements.

Considering the uptake efficiency indices (Table 6) it can be seen that the uptake of most elements per unit root weight increased with decreasing supply. This was especially true for rimu which appeared to have a greater 'uptake ability' than the other two species at low rates of supply. Chapin (1980) states that slowly growing species characteristic of infertile soils usually absorb considerably less nutrients under nutrient-rich conditions than do species from fertile habitats. Under poor conditions however, these infertile-soil species often absorb similar or larger quantities to those species characteristic of fertile sites. This observation appears to be true of rimu which was significantly more efficient in the uptake of nutrients than the other two species at lower rates of supply.

Root absorption capacity (the absorption rate per unit of root under standardized conditions) is usually higher in

species from fertile habitats, especially at moderate and high nutrient availabilities (Chapin, 1980). In the absence of selection pressures in fertile conditions for a high root absorption capacity, a lower capacity with lower protein requirements would probably be favoured.

Plants with high absorption capacities also have high efflux rates (Chapin, 1980); thus it would be selectively advantageous to have a high absorption capacity in fertile sites and a high efficiency on poor sites. Although it was clear that rimu had a greater efficiency in uptake per gram of root than the other two species, it was not obvious that kahikatea or totara had greater root absorption capacities. Even at the top levels of nutrient supply, the concentration of elements in their tissues did not exceed those of rimu by very much, thus the three species may have very similar absorption capacities.

While the uptake per gram of root of most elements decreased with increasing supply, the uptake of calcium increased slightly. This element was added at a constant rate to all nutrient treatments, therefore this trend is most likely a result of the trees' improved absorption capacity in soils of improved fertility (Chapin, 1980). The uptake of calcium may be associated with the uptake of anions, particularly NO_3^-, PO_4^- and SO_4^{2-}, which varied in supply between treatments (D. Mead, pers. comm.). It is not known, however, if the species were utilizing NO_3^- or NH_4^+, or both as a nitrogen source. The fact that the uptake of calcium remained comparatively constant over all rates of supply indicates that active selection was occurring.

INTRODUCTION TO THE SECOND NUTRITION EXPERIMENT

In the first nutrition experiment, the cause of the unusual increase in growth of some totara seedlings in the 10 ppm and 60 ppm treatments was not determined. The increase in size was more than could be attributed to a mistaken addition of nutrients, but Baylis et al. (1963) and Baylis (1969) achieved the same magnitude of response in both totara and kauri when these species were infected with mycorrhizas. Research has shown that a major result of mycorrhizal infection is an increase in the uptake of phosphorus, and often nitrogen (Harley and Smith, 1983). For instance, Bowen (1980) found the concentration of phosphorus in the shoots and roots of hoop pine infected with mycorrhizas to be double that of uninfected plants. In the first nutrition experiment, the phosphorus concentration of the anomalous totara seedlings was twice that of the normal seedlings and their nitrogen content was also higher (Appendix E); therefore it appears highly likely that the extra growth in these totara seedlings was caused by mycorrhizal infection.

Current Knowledge of VA Mycorrhizas in New Zealand

The podocarps all form associations with vesicular-arbuscular (VA) mycorrhizas which, ecologically, are the most important mycorrhizas in New Zealand forests (Harley and Smith, 1983). Some mycorrhizal species known to infect podocarps are: *Acaulospora laevis*, *Sclerocystis rubiformis*, *Glomus fasciculatum*, *Glomus microcarpum*, *Gigaspora* sp. and *Endogone* sp. (Johnson, 1973, Baylis, 1969). VA mycorrhizal infection has been found in totara, kauri (Baylis et al., 1963; Baylis, 1969), kahikatea, Hall's totara (Johnson, 1973), southern rata, manuka (Hall, 1977; Baylis, 1971), kamahi (Hall, 1975), broadleaf (Baylis, 1959; Greenhall, 1963), karamu (Baylis, 1967), many ferns (Cooper, 1975) and tussock species (Crush, 1973).

Mycorrhizal infection can influence competition between plants, as fungal hyphae from an infected plant can exploit a greater soil volume for nutrients, thereby giving it an advantage over an uninfected plant. Competition may also be influenced even if all plants are associated with mycorrhizas. The various species of mycorrhizas differ in their effectiveness, and host species differ in their response.

The effectiveness of a particular species of mycorrhiza can depend on its ability to: develop extensive infection, form an extensive and well-distributed hyphal network, absorb phosphorus from the soil, and maintain the transport mechanism along the hyphae to the roots (Abbott and Robson, 1984). Species and strains of mycorrhizas have shown marked variation in the time required for spore germination, the spread of infection, the development of arbuscles and vesicles, and the growth of hyphae (Bevege and Bowen, 1975).

Plants that are economical in their use of nutrients, or that have high absorption capacities or rooting densities will be affected differently by mycorrhizal infection than plants without these characteristics (Baylis, 1975). Whether a plant species benefits from mycorrhizal infection also depends on plant growth rate and soil phosphate availability (Johnson, 1976). Plant growth rate affects dependence on mycorrhizas as slow-growing plants may have lower nutrient demands, and therefore may not benefit from the extra nutrient uptake provided by the fungi (Johnson, 1976). Soil phosphorus availability can influence infection in some species which only need the extra 'uptake ability' of the mycorrhizas when phosphorus is deficient. For instance, the growth of southern rata is only stimulated by mycorrhizal infection when soil phosphorus is low, whereas kamahi requires mycorrhizal infection for growth in any soil (Hall, 1975). Likewise, the range of soil phosphorus availabilities which will provide a stimulus for the growth of mycorrhizas

in manuka is lower than for karamu (Baylis, 1971). Some evidence indicates that the spread of mycorrhizal infection is limited by the phosphorus concentrations in the host rather than in the soil solution (Sanders, 1975).

Root morphology seems to be a major factor in the potential for the mycorrhizal infection of a species. Baylis (1972) established a relationship whereby plants deficient in root hairs (magnolioid roots) benefit more from mycorrhizal infection than do plants with abundant root hairs. Without mycorrhizas, the plants with magnolioid roots require much higher soil phosphate levels for growth, thus broadleaf and totara require higher levels of available phosphorus than karamu for non-mycotrophic growth (Baylis, 1970).

The mycelium in mycorrhizal, magnolioid roots performs the same function as do the root hairs in other plants, in that the root can effectively occupy a greater soil volume. Growth of up to 80 cm of hyphae per centimeter of infected root has been recorded in onion (Sanders et al., 1977); thus VA mycorrhizas increase plant growth primarily by shortening the distance nutrients must diffuse through the soil to the roots (Abbott and Robson, 1984). Assuming equal specific gravity, 1 mg of hyphae of 10 μm diameter has the same length as 1600 mg of root of 400 μm diameter; therefore compared to roots, hyphae are a highly efficient and energy-conserving mechanism for the absorption of ions of low mobility such as P, Cu, Zn, S and Fe (Bowen et al., 1975).

The podocarps, then, should be highly dependent upon mycorrhizal hyphae as they lack root hairs, and even the lateral roots are often replaced by nodules. These nodules differ in cellular configuration from lateral roots (Khan, 1967), but are perennial, with a new nodule arising at the apex of the old (Baylis et al., 1963). The nodules were originally thought to relate to water storage, but it was also noted that they contained fungi (Yeates, 1924). Later the water storage theory was discounted, but it was found

that phycomycetous fungi were the most prominent organisms in the nodule cortex, and 95 percent of podocarp roots contained endophytic mycelium (Baylis et al., 1963). While the podocarp species form nodules with or without mycorrhizal infection, in non-mycorrhizal plants, the nodules are filled with starch, while in mycorrhizal plants, starch is not conspicuous and abundant phycomycetous hyphae are contained (Baylis et al., 1963).

Scientists have attributed a nitrogen-fixing role to the root nodules of some podocarp species (Bergersen and Costin, 1964; Becking, 1965) and kauri (Morrison and English, 1967). Experiments with 14 New Zealand conifer species however, indicated that while there is enhanced activity of nitrogen-fixing organisms in the region of nodulated roots, these organisms appear to reside in the soil, not within the plant (Silvester and Bennett, 1973). Analysis of South American and Indonesian podocarps also failed to find evidence of nitrogen fixation within the root nodules (Furman, 1970; Becking, 1976).

The primary site for VA mycorrhizas to develop is in the cortical region of the terminal feeder roots which is the most active site for nutrient uptake (Kormanik and McGraw, 1982). When the cortex on the podocarp nodules is renewed each year, it is immediately infected (Baylis, 1972). The same type of process occurs in hoop pine, and in that species, mycorrhizal infection allows the short laterals to continue absorbing nutrients after they are suberized, with the re-infection of new tissue giving the short roots an effective life of two to three years (Bowen et al., 1975). In other plants, as the root matures, the cortex is sloughed off; therefore the repeated cycles of infection peculiar to podocarps and araucarians may be an adaptation for retaining the symbiont after the long roots have shed their cortex (Baylis et al., 1963; Baylis, 1972).

One of the main benefits of mycorrhizal infection is an improvement in the phosphorus status of the infected plant (Harley and Smith, 1983). Water uptake may also be enhanced by the improved nutrient status of a mycorrhizal plant, and infected plants may also be more resistant to disease (Abbott and Robson, 1984; Bagyaraj, 1984). In the first nutrition experiment it could be seen that, compared to the other macro-nutrients, the demand for phosphorus was great in all species. As this element is most often limiting in soils (Baylis, 1975), all species would probably benefit from mycorrhizal infection.

Little has been published on the response of New Zealand podocarps to mycorrhizal infection. Totara and kahikatea have both shown improved growth with mycorrhizal association (Johnson, 1973; Baylis, 1969; Baylis et al., 1963), but investigation of the interaction of nutrition and mycorrhizal infection is limited. Because mycorrhizal association may be important to the nutrition of rimu, kahikatea and totara, and to their interrelationships, a further experiment designed to explore the species' responses to mycorrhizal infection was undertaken.

MATERIALS AND METHODS - SECOND NUTRITION EXPERIMENT

Experimental Design and Conditions

Seeds of rimu, kahikatea and totara were germinated in sterile potting soil in a heated glasshouse one month prior to the start of the experiment. All seed had been kept moist at 4°C before germination. Due to considerable loss of seed viability, it was only possible to represent each species by one provenance in the second experiment. Rimu seed was from Saltwater State Forest, kahikatea seed was from south Westland, and totara seed was from Gwavas State Forest (see Appendix B for details of seed origin).

There were three nutrient treatments and two mycorrhizal treatments within each nutrient treatment - a control and an infected treatment. At the start of the experiment, 30 seedlings of each species were divided into five size classes. The six seedlings in each size class were then randomly allocated each to one of the six treatments, thus there were five seedlings in each treatment. There were no replicates of the treatments due to a shortage of seedlings. The five seedlings in each treatment were planted in sterile vermiculite in two, 16 X 12 cm pots - one containing three seedlings and the other, two. The pots were placed randomly on a glasshouse bench, and their positions were re-randomized every week.

The nutrient concentrations used in the second experiment were identical to those used in the first; however only the 10 ppm, 60 ppm and 200 ppm treatments were included due to the lack of seedlings. Because of time constraints in the second experiment, only 600 ml of the 10, 60 and 200 ppm nutrient treatment solutions listed in Table 1 were added to the pots with three seedlings, and 400 ml to the pots with two seedlings.

The mycorrhizal inoculum was prepared from infected roots of totara seedlings which had been growing in the Rangiora Forest Service Nursery, and from infected roots of kahikatea and rimu trees in Dean's Bush, Christchurch. Infection was ascertained in other roots of those plants through the trypan blue staining method of Phillips and Hayman (1970). It was originally intended that the inoculum should contain both spores and infected root segments. After many hours of searching forest and nursery soils for spores using the methods of Gerdemann and Nicolson (1963) and Sutton and Barron (1972), the attempt to find sufficient numbers of spores was abandoned. Apparently some species of mycorrhiza only fruit at certain times of the year and there can be poor years for spore formation (Hall, 1984). Also, non-sporing

races of mycorrhiza may occur where dense root mats enable infection to spread by hyphal contact from one root to the next without the fungus expending resources on spore formation (Hall, 1984). Thus it is quite possible to find soil samples from New Zealand rainforests with few or no spores, and this seemed to be the case for the soils examined.

Since infected roots and hyphal segments are reputed to re-infect new roots more readily than spores (Hall, pers. comm.; Johnson, 1973; Daniels Hetrick, 1984), the lack of spores was not of great concern. Instead, the mycorrhizal treatment seedlings were inoculated with: 2 ml of infected roots chopped into 3 mm segments, 1/2 ml of soil which had been passed through a 100 μm sieve and might have contained hyphae or spores, and a small amount of agar containing mycorrhizal mycelium which had been grown from infected root segments placed on agar at 25°C in the dark for two weeks. All types of inocula were placed in direct contact with the roots of the seedlings at the time of transplanting into vermiculite.

In order to keep the nutrient additions the same, the control seedlings were inoculated with the same amount of root choppings and soil sievings, but these had been boiled to kill any fungi. The control seedlings were also inoculated with sterile agar and with washings from infected roots filtered through a 3 μm filter, which was intended to remove mycorrhizal spores, but to let bacteria pass through.

The experiment was carried out in a heated glasshouse over 14 weeks of the spring and early summer. The glasshouse temperature averaged 20°C over the duration of the experiment (range 15° to 38°C). Light levels varied with the time of day and season, with maximum light intensity approaching 1000 $\mu molm^{-2}s^{-1}$. Relative humidity also varied throughout the day ranging from 20 to 90 percent.

The nutrient solutions were added in increasing amounts every three weeks as in the first experiment; however a capillary watering system was not used in the second experiment. Concern over the possible spread of mycorrhizal infection to control seedlings through the irrigation water was a major reason the capillary watering system was not used. It was thought that top watering might also prevent nutrient loss from the pots; thus pots were watered with 50 ml of distilled water every 1 1/2 days for the duration of the experiment.

Measurement of Growth and Photosynthesis

The height and number of branches were measured for each seedling at the beginning of the experiment and again every month for the duration.

One day before the experiment was terminated, some seedlings were measured for photosynthetic rate. Three size class pairs of inoculated and non-inoculated seedlings of each species in each treatment were selected randomly and measured using the LI-6200 Portable Photosynthesis System as in the first nutrition experiment. The LI-COR system had been updated between the first and second nutrition experiments, therefore only a 5 ppm drawdown in CO_2 concentration was required to measure net photosynthesis in the second experiment. All photosynthetic measurements were expressed on a total foliage area basis, and leaf areas were measured with a Delta-T Area Meter.

Fourteen weeks after inoculation, the seedlings were carefully removed from the vermiculite and measured for height, number of branches and stem diameter at soil level. The trees were then divided into root, stem and leaf portions and the stems and leaves were oven-dried at 80°C for 48 hours and weighed.

The roots of every seedling were stained for mycorrhizal infection using the trypan blue staining method

of Phillips and Hayman (1970). All roots were then examined for the percentage and intensity of colonization using the non-systematic method outlined by Kormanik and McGraw (1982). After scoring, the roots were oven-dried at 80°C for 48 hours and weighed.

On the basis of the root examinations, the stems and leaves of the seedlings of each species in each nutrient treatment were bulked into two groups - infected and uninfected seedlings. The bulked leaves and stems of all groups were then ground to a fine powder, digested and analyzed for concentrations of nitrogen and phosphorus using the steam distillation and automated methods of Nicholson (1984) respectively. As in the first experiment, this work was conducted in the laboratory of the Forest Research Centre, Christchurch.

Analysis

(i) Growth and Relative Growth Rate. All seedlings were classified as 'infected' or 'uninfected' with mycorrhizas on the basis of the root examinations. For each species, every infected seedling was matched with its uninfected size class partner in that nutrient treatment. Inoculated seedlings that had not become infected were discarded along with their non-inoculated size class partner. Non-inoculated seedlings that had become infected and their size class partner were also discarded.

For the analysis of variance of the measured growth variables: total, root, stem and leaf weights, height, number of branches and diameter, and the ratios of the portion weights to total weight; the General Linear Model Procedure of SAS (1985) was used because there were unequal numbers of observations in each species and nutrient treatment. The model was as follows, assuming no missing observations (see Appendix H for sums of squares):

SOURCE OF VARIATION	df
Species	2
Nutrient Treatment	2
Mycorrhiza Treatment	1
Species X Nutrient	4
Size Class	4
Error	76
Total	89

The 'Error' term was comprised of the following elements:

SOURCE OF VARIATION	df
Species X Mycorrhiza	2
Nutrient X Mycorrhiza	2
Species X Size Class	8
Nutrient X Size Class	8
Mycorrhiza X Size Class	4
Spp. X Nutr. X Mycor.	4
Spp. X Nutr. X Size Class	16
Spp. X Mycor. X Size Class	8
Nutr. X Mycor. X Size Class	8
Spp. X Nutr. X Mycor. X Size Class	16

As the amount of variation explained by these interactions was not significant, the terms were pooled to increase precision.

A separate analysis was done for each species for which the model was:

SOURCE OF VARIATION	df
Nutrient Treatment	2
Mycorrhiza Treatment	1
Nutrient X Mycorrhiza	2
Size Class	4
Error	20
Total	29

(see Appendix I for sums of squares). Again, the interaction terms were not significant and so were pooled in the 'Error' term to increase precision.

A regression of growth on the percentage colonization of the roots by mycorrhizal fungi was calculated for each species and nutrient treatment to test if the degree of infection affected growth.

Relative growth rates for each species in each treatment were calculated in the same manner as the first

experiment (R^2 for natural log total weight/natural log height=0.8 for rimu, 0.6 for kahikatea and 0.9 for totara).

(ii) Photosynthesis. As seedlings of all size class pairs measured for net photosynthesis were subsequently confirmed as being either infected or uninfected, the Analysis of Variance Procedure of SAS (1985) was used to compare photosynthetic rates. The photosynthetic rates of the three species were analyzed separately, and inter-specific comparisons are invalid due to varying leaf morphology and stomatal arrangement. The model was similar to that for the analysis of growth by species (see Appendix J for sums of squares).

(iii) Tissue Analysis. The measured element concentrations in the tissues of infected and uninfected seedlings in each nutrient treatment were compared by analysis of variance. The model was as follows (see Appendix K for sums of squares):

SOURCE OF VARIATION	df
Species	2
Nutrient Treatment	2
Mycorrhiza Treatment	1
Nutrient X Mycorrhiza	2
Error	10
Total	17

The 'Error' term was comprised of the following:

SOURCE OF VARIATION	df
Species X Nutrient	4
Species X Mycorrhiza	2
Spp. X Nutr. X Mycor.	4

As the amount of variation explained by these interactions was not significant, the terms were pooled.

The uptake efficiency index was calculated for infected and uninfected seedlings of each species in each nutrient treatment to investigate whether mycorrhizal infection improved uptake ability. This index was calculated in the same manner as in the first nutrition experiment.

RESULTS OF THE SECOND NUTRITION EXPERIMENT

Infection

Mycorrhizal infection was established successfully after 14 weeks in all of the inoculated rimu and kahikatea seedlings. In these species, the non-inoculated seedlings remained uninfected. All but one of the inoculated totara seedlings developed mycorrhizal infection, and the seedling that remained uninfected was the smallest seedling in the 10 ppm nutrient treatment. Most of the non-inoculated totara seedlings remained uninfected, however two seedlings in both the 60 ppm and 200 ppm non-inoculated nutrient treatments developed small amounts of mycorrhizal infection. The results from these five size class pairs of totara seedlings were discarded before analysis.

In all species, mycorrhizal infection appeared to be best established in the upper, older portions of root. There was no significant difference in the percentage or intensity of colonization between species. Infection appeared to be best developed in totara and kahikatea however, as vesicles and arbuscles were present in the infected roots of these species. These structures were very scarce in rimu roots, and infection consisted primarily of extensive hyphal networks. This could also indicate that the rimu seedlings were infected by a different species of mycorrhiza. No external signs of infection could be seen.

The percentage of mycorrhizal colonization was significantly ($p=0.05$) affected by nutrient treatment in kahikatea. Kahikatea seedlings showed infection decreasing from an average of 70 percent in the 10 ppm treatment to 34 percent in the 200 ppm treatment. Totara seedlings displayed the same trend with infection decreasing from 62 to 37 percent, however this was not statistically significant. Rimu seedlings demonstrated the opposite response, with the highest percentage of infection (66 percent) occurring in the

200 ppm nutrient treatment, and the lowest percentage infection (50 percent) in the 10 ppm treatment. This difference was not significant.

No relationships could be found in any of the species between the percentage of mycorrhizal colonization and growth.

Growth and Relative Growth Rate

In all species, growth was influenced by species, nutrition, and mycorrhizal infection (Plate 6). Growth of all measured variables for the three species was significantly different ($p=0.0001$). On average, the kahikatea seedlings were the tallest of the three species. They also averaged the greatest number of branches, diameter at soil level, and stem weight. The totara seedlings averaged the greatest leaf and root weights and therefore the greatest total weight. The rimu seedlings were the smallest on average for all measured growth variables. Nutrient treatment also had a significant ($p=0.001$) effect on the growth of all measured variables in all species, with increased growth resulting from improved nutrition in both mycorrhizal and non-mycorrhizal seedlings. Seedlings infected with mycorrhizas had significantly ($p=0.05$) greater growth for all measured variables except root weight.

The nutrient treatment X species interaction term was significant in the analysis of all measured growth variables, therefore separate analyses by species were made (Table 7).

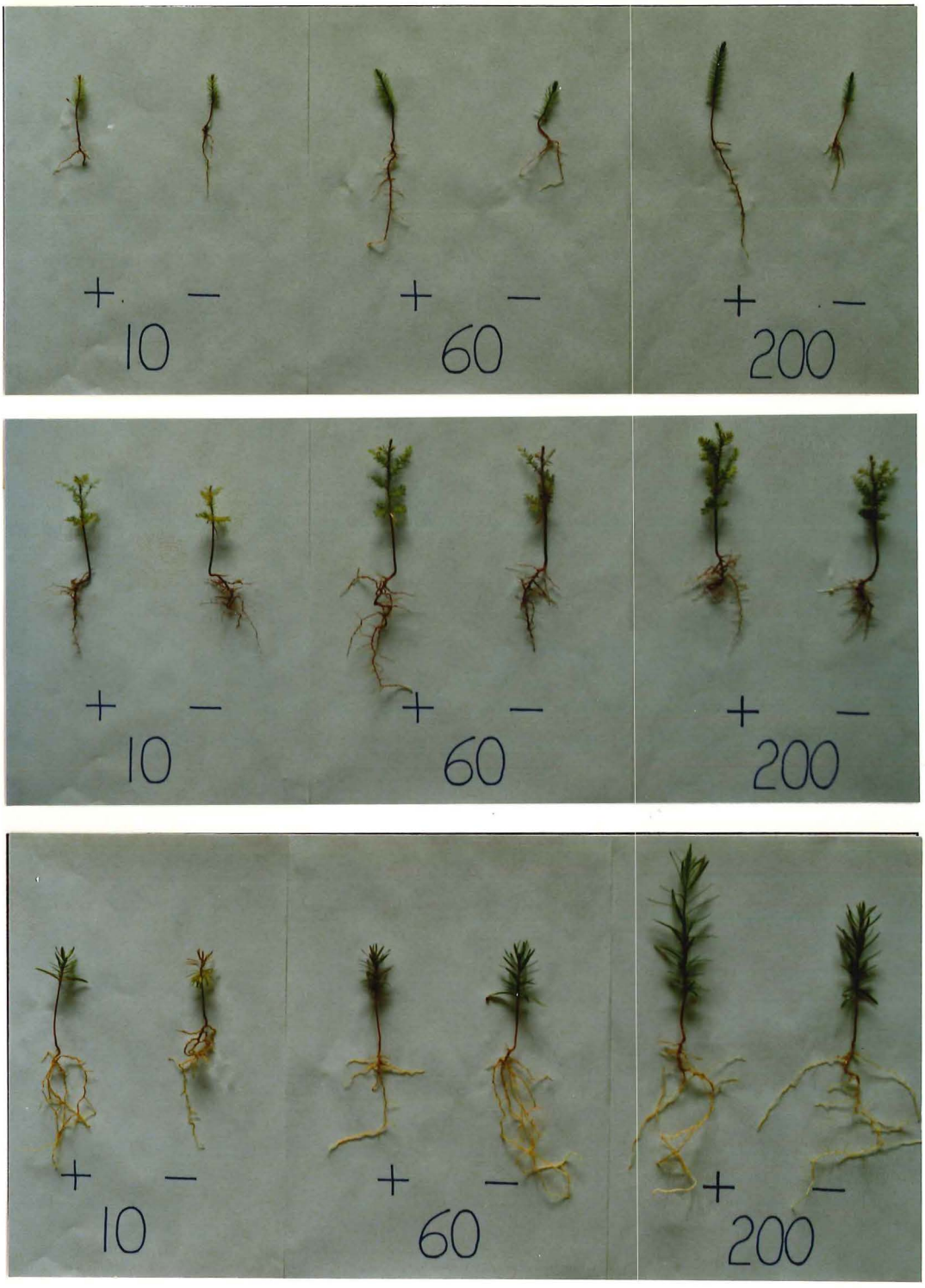

Plate 6: Average whole trees of rimu (top), kahikatea (centre) and totara (bottom) infected (+) and uninfected (-) with mycorrhizas from three nutrient treatments

Table 7: Measured growth variables in the second nutrition experiment

Nutrient Treatment	Rimu +	Rimu -	Kahikatea +	Kahikatea -	Totara +	Totara -
TOTAL WEIGHT (g)						
200	0.0316	0.0192*	0.1229	0.0977	0.2286	0.1894
60	0.0256	0.0149*	0.0914	0.0704	0.0923	0.0868
10	0.0156	0.0150	0.0586	0.0498**	0.0716	0.0549
Mean	0.0243	0.0158**	0.0910	0.0726**	0.1249	0.1048
LEAF WEIGHT (g)						
200	0.0139	0.0099	0.0471	0.0329*	0.1099	0.0732
60	0.0127	0.0069*	0.0256	0.0212	0.0501	0.0391
10	0.0069	0.0067	0.0163	0.0136**	0.0307	0.0219*
Mean	0.0112	0.0076**	0.0297	0.0226**	0.0603	0.0425
ROOT WEIGHT (g)						
200	0.0109	0.0045*	0.0405	0.0368	0.0797	0.0906
60	0.0082	0.0047	0.0414	0.0293	0.0293	0.0368
10	0.0039	0.0041	0.0265	0.0225	0.0304	0.0237
Mean	0.0077	0.0039**	0.0362	0.0295	0.0449	0.0477
HEIGHT (mm)						
200	35.8	22.4**	54.4	46.0	81.0	43.0
60	29.0	22.0	44.0	34.2**	23.0	20.0
10	20.6	19.6	25.8	19.4*	17.2	14.0
Mean	28.5	21.3**	41.4	33.2**	38.1	24.5
DIAMETER (mm)						
200	0.71	0.65	1.08	1.06	1.02	0.95
60	0.70	0.57	1.00	0.93	0.78	0.70
10	0.66	0.57*	0.92	0.86	0.72	0.70
Mean	0.69	0.60**	1.00	0.95*	0.83	0.77

\+ Average for seedlings infected with mycorrhizas
\- Average for seedlings not infected with mycorrhizas
* Difference between + and - value significant (p=0.05)
** Difference between + and - value significant (p=0.01)

For all three species regardless of mycorrhizal treatment, nutrient treatment had a significant effect (p=0.05) on seedling growth in total weight, all portion weights, and height; with an increasing nutrient supply improving growth. Improving nutrition also significantly (p=0.05) increased branch number in rimu and kahikatea, and

diameter in kahikatea and totara. As in the first nutrition experiment, the totara seedlings appeared most responsive to improved nutrition, with uninfected seedlings averaging a 245 percent increase in total weight between the 10 and 200 ppm nutrient treatments. Kahikatea was the next most responsive with a 96 percent increase in the total weight of uninfected seedlings, followed by rimu (28 percent).

For rimu and kahikatea, mycorrhizal infection resulted in significantly ($p=0.05$) improved growth for all measured variables except root weight. For totara, the same trend was obvious for most measured variables, however the differences were not significant ($p=0.20$) due to the limited number of seedling pairs. Mycorrhizal infection appeared to make no difference to the root weight of totara seedlings.

Rimu appeared to have the greatest response in total weight to mycorrhizal infection, particularly in the higher nutrient treatments. This species showed a 64 percent increase in the growth of infected, compared to uninfected, seedlings in the 200 ppm treatment; while kahikatea showed a 26 percent increase and totara, a 21 percent increase.

Rimu was the only species to show a significant nutrient treatment X mycorrhiza treatment interaction in analyses of variance of total weight, root weight and height. Kahikatea and totara had a similar degree of response to improved nutrition whether or not they were infected with mycorrhizas. Rimu however, showed a mere 28 percent improvement in total weight between the 10 and 200 ppm nutrient treatments in uninfected seedlings, but a 102 percent improvement in growth over the same nutrient range in mycorrhizal seedlings.

The graph of relative growth rate (Figure 5) shows that the significant nutrient treatment X mycorrhizal treatment interaction in rimu may have been due to starting size effects.

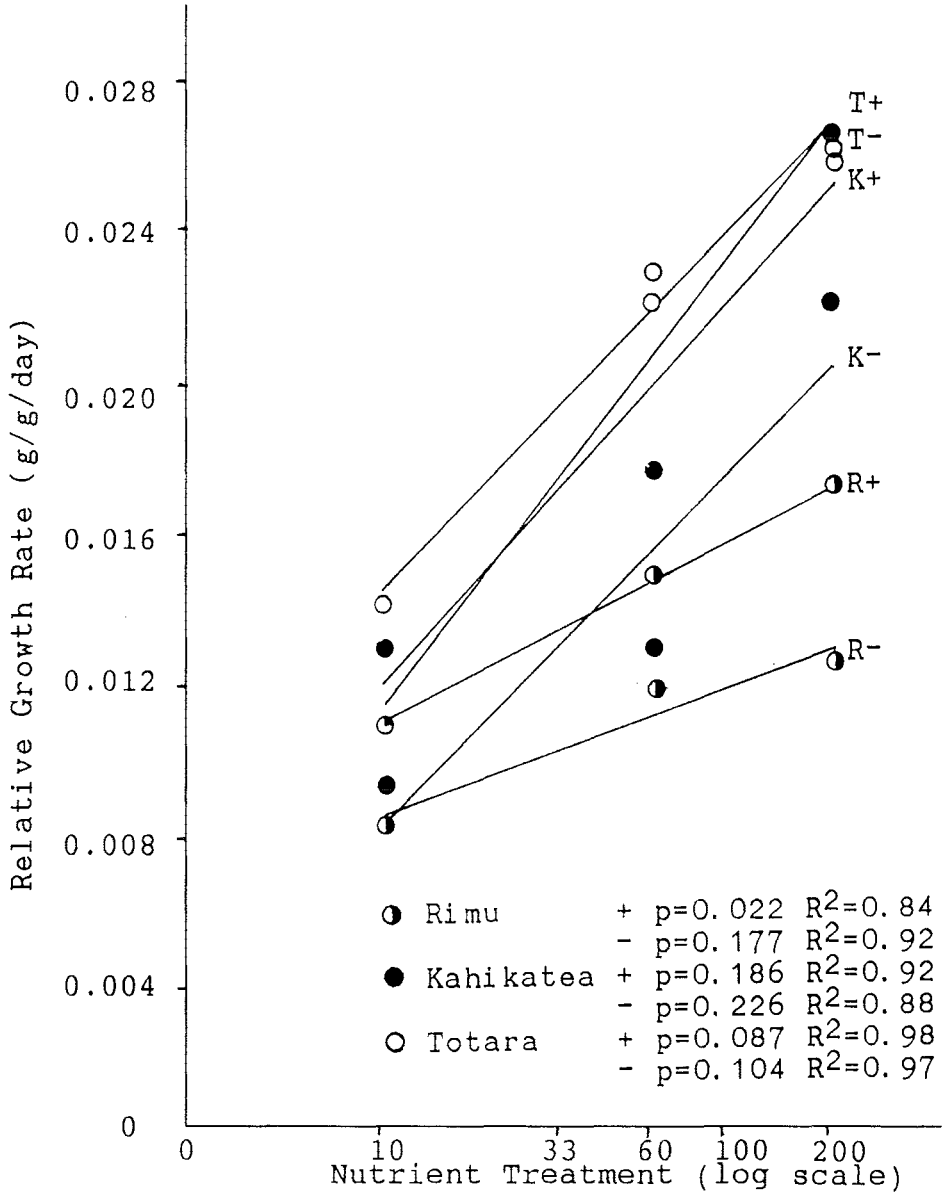

Figure 5: Plot of relative growth rate for three species in three nutrient treatments, with (+) and without (-) mycorrhizal infection (estimated starting weights)

Relative growth rates of both rimu and kahikatea showed a uniform response to mycorrhizal infection, with the improvement in growth rate of infected seedlings being approximately the same in all nutrient treatments. This contrasted with the response of the totara seedlings which showed their greatest improvement in relative growth rate with mycorrhizal infection at the lowest level of nutrition. This improvement became almost non-existent in the 200 ppm nutrient treatment.

Allocation of Resources

The trends of allocation of resources between the three species were similar to those in the first nutrition experiment. On average, totara allocated the most resources to leaf growth and kahikatea, to root growth regardless of mycorrhizal infection (Table 8).

Table 8: Leaf weights and root weights as a proportion of total weight in the second nutrition experiment

Nutrient Treatment	Rimu +	Rimu -	Kahikatea +	Kahikatea -	Totara +	Totara -
LEAF WEIGHT/TOTAL WEIGHT						
200	0.4496	0.4981*	0.3830	0.3398**	0.5035	0.4310
60	0.5119	0.4733	0.2825	0.2986	0.5460	0.4536
10	0.4461	0.4556	0.2799	0.2747	0.4285	0.4002
Mean	0.4692	0.4814	0.3151	0.3044	0.4863	0.4255**
ROOT WEIGHT/TOTAL WEIGHT						
200	0.3316	0.2302**	0.3290	0.3698	0.3338	0.4229
60	0.2980	0.2553	0.4495	0.4003	0.3155	0.4090
10	0.2420	0.2659	0.4524	0.4471	0.4229	0.4304
Mean	0.2906	0.2368*	0.4103	0.4057	0.3640	0.4218*

\+ Average for seedlings infected with mycorrhizas
\- Average for seedlings not infected with mycorrhizas
* Difference between + and - value significant (p=0.05)
** Difference between + and - value significant (p=0.01)

There was no significant difference between nutrient treatments for leaf weight ratio in rimu, however increasing nutrition significantly ($p=0.02$) increased this ratio in kahikatea and totara. As in the first nutrition experiment, improved nutrition tended to result in decreasing root weight ratios, however the effect of nutrient treatment on this variable was significant only for kahikatea ($p=0.001$).

Mycorrhizal infection had a significant influence on leaf weight ratios only in totara ($p=0.01$) where infected seedlings allocated more resources to leaves than uninfected seedlings. Rimu and kahikatea showed no consistent changes in leaf weight ratio due to mycorrhizal infection.

The ratio of root weight to total weight was significantly ($p=0.05$) influenced by mycorrhizal infection in both rimu and totara, however in an opposite manner. Rimu showed an increased allocation of resources to infected roots, particularly at higher levels of nutrition. Totara, however, had a decreased allocation of resources to infected roots. Kahikatea tended to have an increased root weight ratio in infected seedlings, but this trend was not significant.

Photosynthesis

The photosynthetic rates of the three species are not comparable due to differing leaf morphologies and stomatal arrangements. As in the first nutrition experiment, nutrient treatment had a significant ($p=0.003$) influence on the photosynthetic rates of all three species (Table 9) with improved nutrition resulting in higher photosynthetic rates. All three species also showed a significant ($p=0.001$) increase in net photosynthetic rate in seedlings infected with mycorrhizas.

Table 9: Mean rates of net photosynthesis ($mgCO_2/m^2s$) in the second nutrition experiment

Nutrient Treatment	Rimu +	Rimu -	Kahikatea +	Kahikatea -	Totara +	Totara -
200	0.407	0.258**	0.607	0.399**	0.567	0.557
60	0.386	0.280**	0.551	0.314**	0.606	0.370**
10	0.242	0.240	0.353	0.184*	0.402	0.207**
Mean	0.345	0.260**	0.504	0.299**	0.502	0.380**

\+ Average for seedlings infected with mycorrhizas
\- Average for seedlings not infected with mycorrhizas
* Difference between + and - value significant (p=0.05)
** Difference between + and - value significant (p=0.01)

In both kahikatea and totara, the increase in photosynthetic rate in seedlings infected with mycorrhizas declined with improving nutrition from almost 100 percent in the 10 ppm treatment, to 52 percent in the 200 ppm treatment in kahikatea and 2 percent in totara. Rimu demonstrated the opposite trend however, with the increase in photosynthetic rate in mycorrhizal seedlings becoming larger at higher nutrient treatments.

Tissue Analysis and Nutrient Uptake

Concentrations of nitrogen and phosphorus in non-mycorrhizal seedlings followed similar patterns to the first nutrition experiment. Nutrient treatment had a significant (p=0.002) effect on the concentration of the two elements in the seedlings, with higher concentrations resulting from greater additions (Table 10). At lower nutrient concentrations, all species appeared to have a greater efficiency of uptake (Table 10); and at these lower concentrations, rimu appeared to have a higher uptake per unit weight (Table 10) and uptake efficiency (Table 11) than the other two species.

When infected with mycorrhizas, seedlings of all species had significantly (p=0.02) higher phosphorus

concentrations in their stem and leaf tissues than did uninfected seedlings (Table 10). Infected seedlings of all species also were much more efficient in the uptake of phosphorus per unit root weight (Table 11).

Table 10: Percentage concentrations of nitrogen and phosphorus in seedling shoots in the second nutrition experiment

Nutrient Treatment	Rimu +	Rimu -	Kahikatea +	Kahikatea -	Totara +	Totara -
NITROGEN						
200	1.70	1.34	1.60	1.46	1.64	2.53
60	1.49	1.21	1.33	1.09	1.24	1.30
10	0.93	1.02	0.94	0.83	0.85	0.81
Mean	1.37	1.19	1.29	1.13	1.24	1.55
PHOSPHORUS						
200	0.38	0.18	0.20	0.15	0.28	0.22
60	0.23	0.20	0.18	0.12	0.18	0.14
10	0.20	0.17	0.14	0.10	0.15	0.11
Mean	0.27	0.18*	0.17	0.12*	0.20	0.16*

\+ Average for seedlings infected with mycorrhizas
\- Average for seedlings not infected with mycorrhizas
* Difference between + and - value significant (p=0.05)

Seedlings infected with mycorrhizas did not have significantly different nitrogen concentrations in their shoot tissues from uninfected seedlings. Mycorrhizal seedlings of kahikatea had increased nitrogen concentrations and increased nitrogen uptake efficiency in all nutrient treatments. Infected totara seedlings showed increased nitrogen content only in the lowest nutrient treatment, however nitrogen uptake efficiency was greater in mycorrhizal seedlings in both the 10 ppm and 60 ppm nutrient treatments. For rimu, the concentration of nitrogen in mycorrhizal seedling tissues was higher than in non-mycorrhizal seedlings in the 60 ppm and 200 ppm nutrient treatments, but uptake efficiency was greater in the 10 ppm and 60 ppm treatments.

Table 11: Uptake efficiency indices in the second nutrition experiment

Nutrient Treatment	Rimu +	Rimu -	Kahikatea +	Kahikatea -	Totara +	Totara -
NITROGEN						
200	123.1	142.2	121.5	97.3	117.7	132.2
60	387.8	321.3	244.4	217.7	326.3	256.5
10	1871.8	1865.8	1045.3	917.8	1001.6	936.7
PHOSPHORUS						
200	91.7	75.5	50.3	34.3	66.2	37.7
60	197.8	180.2	113.4	77.4	159.3	95.1
10	1307.7	1036.6	530.8	354.1	603.0	407.9

\+ Average for seedlings infected with mycorrhizas
\- Average for seedlings not infected with mycorrhizas

DISCUSSION OF THE SECOND NUTRITION EXPERIMENT

VA mycorrhizas infect more plant species and are more widely distributed geographically than any other type of mycorrhiza (Gerdemann, 1975). While ectomycorrhizas are most common in cool regions of the globe, temperate regions have both ecto- and VA mycorrhizas, and tropical plants are almost exclusively associated with VA mycorrhizas (Gerdemann, 1975). This distribution probably occurs because most ectomycorrhizas have an optimum temperature for growth far below that of VA mycorrhizas (Redhead, 1980).

The world-wide distribution of VA mycorrhizas may be accounted for by the great antiquity of this group of fungi (Redhead, 1980). The earliest evidence of VA-type mycorrhizal infection has been found in strata approximately 370 million years old. While the plants of that time did not possess true roots, they were clearly infected by fungi very similar to today's VA mycorrhizas (Harley and Smith, 1983). Many gymnosperm fossils with mycorrhizas similar to VA mycorrhizas have been identified from Carboniferous deposits (Nicolson, 1975).

Although VA mycorrhizas have been associated with plant species for millions of years, co-evolution has not resulted in the specialization of the fungi (Harley and Smith, 1983). They have only speciated slowly, and have remained cosmopolitan, with each fungus species infecting a wide range of hosts (Johnson, 1973). Hosts may also associate with more than one species of mycorrhiza at one time (Harley, 1985). This would imply that for VA mycorrhizas, the benefits of developing relationships with specific hosts must have been offset by the advantage in dispersal of using every root as a "staging post" (Baylis, 1975). The higher fungi, including ectomycorrhizas, evolved later and overcame dispersal problems by developing airborne spores; thus they have developed host specificity. It is ectomycorrhizas that form associations with the Pinaceae, while the coniferous families of the southern hemisphere remain in association with the more primitive VA mycorrhizas.

Many of the New Zealand podocarp species have been shown to form associations with mycorrhizal fungi, and to benefit from these associations (Baylis et al., 1963; Baylis, 1969; Johnson, 1973). In this experiment, rimu, kahikatea and totara seedlings grown at three levels of nutrition all became infected with mycorrhizal fungi and showed improvements in growth and nutrient status after infection.

Infection

The general success of infection may have been due to the optimum temperature and light conditions for fungal growth present in the glasshouse. Mycelial colonization is maximized at 28^{0} to 34^{0}C (Daniels Hetrick, 1984), and temperatures in the glasshouse reached 38^{0}C at the beginning of the experiment. Increased light intensity and duration also increases colonization (Daniels Hetrick, 1984), and the experiment was carried out over three months of the early summer when day length was near maximum. Light intensity

should have been adequate as Baylis (1975) found that manuka, which is only moderately mycotrophic, still responded to mycorrhizal infection when its growth rate was reduced by two thirds due to shading. Effective, stable, mycorrhizal infection is usually achieved five to six weeks after inoculation (Cooper, 1984), so the duration of this experiment was appropriate.

The failure to achieve infection in one inoculated totara plant may have been due to an insufficient dose of inoculum (Cooper, 1984), to its lack of carbohydrates, or to its small root system.

Totara seedlings appear to be much more prone to infection by mycorrhizal fungi than do rimu or kahikatea seedlings, as in both the first and second nutrition experiments, non-inoculated plants of totara became infected with mycorrhizas. Mycorrhizal spores on the seed coats or in the potting soil used for germination could have infected these seedlings, but as totara was not treated any differently from rimu or kahikatea, it would seem to develop mycorrhizal associations more readily than the other species.

The better development of vesicles and arbuscles in the roots of kahikatea and totara could indicate that they were infected by a more efficient species of mycorrhiza which became established more rapidly than the mycorrhizas in rimu roots. On the other hand, it is more likely to indicate the superior carbohydrate status of the two species which would provide the resources for faster mycorrhizal growth.

Maximum mycorrhizal colonization should occur in soils of low fertility (Daft and Nicolson, 1969) and both phosphorus and nitrogen will reduce infection if they are present at high concentrations (Daniels Hetrick, 1984). For this reason, it was expected that the colonization of mycorrhizas would be more intense in the 10 ppm seedlings than in the 200 ppm seedlings, and this was true for infected kahikatea and totara seedlings. Rimu showed the opposite

response, however in this species, the difference in percentage infection between nutrient treatments was much smaller than in the other two species, and the differences were not significant. This could indicate that rimu is more mycotrophic than the other two species, and requires a higher level of nutrient supply before infection is reduced.

Growth and Relative Growth Rate

Mycorrhizal infection significantly improved the growth of rimu, kahikatea and totara seedlings in all nutrient treatments. Mycorrhizas commonly improve the growth of plants with which they are associated, and the major increase in growth is usually in the leaf and stem portions of the plants (Bowen et al., 1975). This was the case for most infected seedlings in the experiment. Accompanying the increased shoot growth, however, was an increase in root weight in most mycorrhizal plants. Infected rimu seedlings had a significant ($p=0.0009$) increase in their root weights as well as shoot weights compared to uninfected seedlings, and this trend was also obvious, if not significant, in kahikatea seedlings and totara seedlings in the 10 ppm nutrient treatment. Improved root growth as a result of mycorrhizal infection has been demonstrated in many host plants including trees (Harley and Smith, 1983) as infected roots tend to have greater lengths, diameters and increased branching compared to uninfected roots (Cooper, 1984).

Increased growth in mycorrhizal plants may be due to several factors, but the primary factor is improved phosphorus nutrition (Harley and Smith, 1983). Where phosphorus availability does not limit plant growth, mycorrhizal infection often depresses growth (Cooper, 1984). This depression is probably due to competition for photosynthate between the plant and fungus, as the biomass of VA mycorrhizas can be up to 17 percent of dry root weight (Abbott and Robson, 1984; Bowen et al., 1975). Where

phosphorus is limiting, growth depression is usually only temporary until the mycorrhiza gets established, and then growth accelerates (Cooper, 1975).

As the growth of all seedlings was improved by mycorrhizas even in the 200 ppm nutrient treatment, phosphorus availability may have been below an optimum level in all nutrient treatments. The infected rimu seedlings in the 10 ppm treatment may have had such a limited improvement in growth because of starting size effects or early growth depression associated with infection. Host growth depression during the infection phase has been attributed to: a pathogenic stage in establishment, competition between the host and fungus for phosphorus or carbohydrates, or phosphorus toxicity (Cooper, 1984). The effects of this depression may be long-lasting, and the rimu seedlings from the 10 ppm nutrient treatment, where carbohydrate and phosphorus supplies would have been most limited, might still have been showing the effects of such a growth depression.

The graph of relative growth rate (Figure 5) shows similar trends to Figure 4 of the first nutrition experiment in that totara had the highest relative growth rates followed by kahikatea and then rimu. The relative growth rates in the second experiment were higher than those in the first probably because the seedlings used were younger, and relative growth rate has been found to decline with age (Sweet and Wareing, 1968). The same rationale may also explain why the growth rates of the totara seedlings in the second experiment were relatively higher than those of the rimu and kahikatea seedlings compared to the first experiment. The totara seedlings germinated almost a month later than the other two species, thus a comparison of relative growth rates between the species should be based primarily on the results of the first nutrition experiment.

Figure 5 indicates that there may be some species' differences in response to mycorrhizas. Totara seems to be

less mycotrophic than the other two species, as the improvement in relative growth rate due to mycorrhizal infection declined at higher levels of nutrition. It appears that a nutrient treatment much richer in phosphorus would be needed before rimu and kahikatea would no longer benefit from mycorrhizal infection.

Allocation of Resources

Mycorrhizas generally increased leaf weight ratios in totara and this response is common to many species (Harley and Smith, 1983). In the 60 ppm nutrient treatment, rimu increased its leaf weight ratio with mycorrhizal infection; however in the 200 ppm treatment, the increase in root weight was so large in infected seedlings that their leaf weight ratio was less than that of uninfected seedlings. Mycorrhizal kahikatea seedlings had substantially larger leaf weight ratios only in the 200 ppm nutrient treatment.

In totara, the allocation of resources to roots decreased with mycorrhizal infection. This trend occurs in other species (Bowen et al., 1975), and was also evident in kahikatea seedlings in the 200 ppm nutrient treatment. Root weight ratios are usually lower in plants grown with adequate supplies of phosphorus than in plants from phosphorus-deficient soils (Hall, 1975). Infected rimu seedlings and kahikatea seedlings from the lower nutrient treatments may not have shown a similar response in root weight ratio due to their slow growth rates. If increased resource allocation to roots was the first response to mycorrhizal infection, these seedlings may have not had enough time to increase their shoot growth enough to alter the root weight ratios.

Rimu again showed the least variation in the apportionment of resources between nutrient treatments, but a significant result of mycorrhizal infection appeared to be an improvement in root growth. It is likely that once infection had become well established in seedlings in the 10 ppm

nutrient treatment, the root weight ratios of infected seedlings would have increased.

Photosynthetic Response

The stomatal physiology of mycorrhizal plants may be affected by infection, and decreased resistance to CO_2 and water movement can result in increased photosynthetic and transpiration rates (Harley and Smith, 1983). Roots infected with mycorrhizas tend to expend more energy than non-mycorrhizal roots as there is a net flux of carbohydrates to the fungus (Lewis, 1975) and a 74 percent increase in CO_2 loss from the roots can result from mycorrhizal infection (Cooper, 1984). This loss must be met by an increase in photosynthesis (Abbott and Robson, 1984).

In the second nutrition experiment, net photosynthesis was increased in seedlings of all three species in all nutrient treatments when infected with mycorrhizas. The increase in photosynthesis was correlated with the percentage infection, thus more heavily infected seedlings tended to have higher rates of net photosynthesis.

Photosynthetic rates of uninfected seedlings were substantially higher in the second experiment than in the first, and this could be due to several factors. The LI-6200 Portable Photosynthesis System had been markedly improved between experiments, and its increased efficiency and accuracy may have resulted in the differences between experiments. The light intensities under which photosynthetic measurements were made were higher in the second experiment, and the seedlings were also much younger. Both of these factors would result in increased rates of net photosynthesis (Sweet and Wareing, 1968), thus the differences in photosynthetic rates between experiments could have been due to one or a combination of these factors.

Tissue Analyses and Uptake Response

The major effect of mycorrhizal infection on the nutrient status of seedlings of the three species was an improvement in the uptake of phosphorus. Without exception, mycorrhizal seedlings had higher concentrations of phosphorus in their shoot tissues and also had a higher uptake efficiency for phosphorus. Totara had the greatest increase in phosphorus uptake efficiency due to mycorrhizal infection followed by kahikatea and then rimu.

In mycotrophic plants, infection always results in increased uptake of phosphorus as mycorrhizal roots absorb 1.5 to 6.0 times more phosphorus than roots of uninfected plants (Cooper, 1984). Mycorrhizal root nodules of kauri were found to have twice the rate of phosphate absorption of non-mycorrhizal nodules (Morrison and English, 1967). Mycorrhizas take up phosphates from the soil, accumulate them as granules of polyphosphate and transfer them by cytoplasmic streaming along the hyphae to the arbuscles. Here the granules are broken down and the phosphorus released to the plants (Bonfante-Fasolo, 1984). The average arbuscle lives for four days, and the phosphorus transfer occurs across the living membrane (Cooper, 1984). Uptake in non-mycorrhizal roots may also be stimulated by the presence of mycorrhizas in other roots of the same plant (Bowen et al., 1975).

Mycorrhizal infection often results in increased uptake of other elements such as Zn, Cu, S and Fe which are transported to the root by diffusion (Abbott and Robson, 1984). Increased Zn uptake has been found in hoop pine roots (Bowen et al., 1975); but in another species, the molar amounts of P, S, and Zn transported over 10 days by the hyphae of Glomus mosseae were 35:6:1. This demonstrates the high efficiency of uptake and translocation of phosphorus compared to other elements by this species of mycorrhiza (Cooper, 1984).

In other plant species, once phosphorus nutrition is improved and growth increases, interpreting the uptake of other macro-nutrients becomes difficult. In some cases, infected plants have a lower percentage of nitrogen in their tissues due to increased biomass. In other situations, the percentage nitrogen may increase due to improved uptake (Cooper, 1984; Harley and Smith, 1983). In the second nutrition experiment, mycorrhizal infection appeared to improve either the nitrogen concentration or nitrogen uptake efficiency of seedlings of all species in all nutrient treatments, except totara seedlings in the 200 ppm treatment. As phosphorus was most likely to be the element limiting growth, mycorrhizal totara seedlings in the highest nutrient treatment may have increased their dry weight to such an extent that the percentage nitrogen content of their tissues was reduced (Cooper, 1984).

In natural soils, the source of nitrogen is often predominantly ammonium rather than nitrate; and as ammonium ions are relatively immobile in soil, mycorrhizas could aid the host plant considerably in nitrogen uptake (Cooper, 1984). Because plants have such high demands for nitrogen, even nitrate, which diffuses much more rapidly through the soil than ammonium, may become deficient in areas near the root (Harley and Smith, 1983). For these reasons, nitrogen uptake should be improved by mycorrhizal infection in the podocarps.

Due to financial and time constraints, nitrogen and phosphorus were the only elements analyzed in the second nutrition experiment. The concentrations of these elements in seedling tissues were higher in the second experiment than in the first, and this may be because the seedlings were harvested earlier in the second nutrition experiment. In the first experiment, the seedlings continued growing for 11 weeks after the final nutrient addition, thus nutrient concentrations would have decreased as dry matter increased.

In the second experiment, the seedlings were harvested soon after the fifth nutrient addition, therefore the concentrations of elements in their tissues would not have been diluted by dry matter increase. In the second experiment, only the leaves and stems were analyzed for nutrient concentrations, whereas roots were included in the first analysis. Nutrient concentrations tend to be higher in shoot tissues (Mead, 1984), therefore this could also have caused the differences. Nutrient additions from the decomposing inocula could have raised tissue element concentrations; and top watering may have prevented leaching losses in the second experiment. Thus there could be many factors contributing to the elevated nutrient concentrations in the second nutrition experiment.

SUMMARY OF THE TWO NUTRITION EXPERIMENTS

While all three species in the first experiment had a capacity for increased growth with improved nutrition, it appeared kahikatea and totara were better able to respond than rimu. Rimu, however, seemed to be more 'competitive' for available nutrients and had a greater efficiency of uptake per unit root weight than the other two species. Although totara is capable of tolerating nutrient-poor sites, rimu is acknowledged to be particularly tolerant, and this may be at least partially due to its characteristics of slow growth and high uptake efficiency.

Soils deficient in nutrients are common and widespread, and the plants that tend to occupy such sites are those which are less disadvantaged by infertile conditions (Epstein, 1972). Species differ in their efficiencies of nutrient absorption and utilization; and slight differences in physiological adaptations will often be amplified by competition to a point where less well-adapted plants are eliminated (Epstein, 1972). Thus rimu, as a slow-growing

species with a high uptake efficiency, may be well adapted to infertile sites, as any available nutrients can be taken up readily but will only be utilized slowly (Chapin et al. 1983).

The second nutrition experiment showed the growth of all three species to be significantly increased with mycorrhizal infection. Rimu appeared to reap the greatest benefit in growth from infection, and its superior uptake efficiency was enhanced by mycorrhizas. Both rimu and kahikatea seemed to be more mycotrophic than totara. Totara, however, had a much greater increase in uptake efficiency for phosphorus when infected with mycorrhizas than the other two species. It also appeared better able to respond in terms of resource allocation, and was able to divert more photosynthate to shoot growth in infected seedlings than rimu or kahikatea.

Competition between species may be significantly affected by mycorrhizal infection, and the effects are probably greater in seedlings than in mature plants (St. John and Coleman, 1983). Several hosts can associate with the same mycorrhiza, and competition between hosts is likely to reside in the process of extracting the most from, and giving the least to, the fungal system (Harley and Smith, 1983). Rimu's high efficiency of uptake may result in an even greater degree of competitiveness when this species is associated with mycorrhizas. As totara appears to be less dependent on mycorrhizas than rimu and kahikatea, it may be more competitive on soils that are without VA mycorrhizas.

Rimu's apparent competitive advantage in nutrient-poor conditions and its significant improvement in growth with mycorrhizal infection might help to explain this species' dominance in certain situations, but not over much of New Zealand. While it could be argued that rimu's efficient uptake of nutrients and slow growth enable it to dominate on the leached and degraded soils of the West Coast of the South

Island, it does not explain this species' dominance on the richer soils of other areas. It therefore appears necessary to look further to explain the site preferences and dominance patterns of rimu, kahikatea and totara.

CHAPTER III

GERMINATION EXPERIMENT

INTRODUCTION

The nutrition experiments provided some insight into the sensitivity of response of rimu, kahikatea and totara to soils of different fertilities; however much more information is needed to explain the ecological distribution of the three species. Temperature was the second environmental factor chosen with which to compare the physiology of the three species under controlled conditions; and an experiment designed to discover the optimum seed germination temperature for the species was considered a logical starting point for this investigation.

There are certain universal conditions which must be met in order to achieve germination in seed of any species (Toole et al., 1956). Moisture for rehydration, oxygen for respiration and temperatures within a suitable range must be present to trigger the germination process.

Germination of the seed of other tree species has proven to be significantly affected by temperature. Species' temperature requirements for germination vary greatly, but it seems that the genetic differences in seed physiology are often an adaptation to climatic contrasts (Stearns and Olson, 1958). Optimum germination temperatures for different tree species vary widely from as low as 12°C for eastern hemlock (Stearns and Olson, 1958) to 35°C for Eucalyptus camaldulensis (Grose and Zimmer, 1958) and Eucalyptus microtheca (Doran and Boland, 1984). The rapid and complete germination of these Eucalyptus species at relatively high temperatures is thought to be an adaptation to the climatic conditions prevailing during seed fall (Doran and Boland, 1984).

Optimum temperatures may also vary between populations of a given species, and even between trees in a stand (USDA, 1974). Some species have broad temperature optima, whilst others have very discrete requirements. Jack pine, for instance, germinates equally well at temperatures between 15° and 30°C; while western hemlock has the greatest germination at 20°C (USDA, 1974).

The great variability of the temperature requirements among and within different species may be influenced by many factors. Seed age, ripeness at harvest, length of storage and storage conditions may all affect the temperature optima for germination of any species (Toole et al., 1956).

Light may also alter the most favourable temperature for germination (Stearns and Olson, 1958); and while this factor was not included in the study, experiments carried out in the late 1950's by D.S. Preest (unpublished) indicated that light intensity had no effect on germination of rimu seed (J.C. van Dorsser, pers. comm.).

Other germination experiments with rimu include M.H. Bannister's 1955 trial at the Forest Research Institute, Rotorua (unpublished). In this trial, stratified and unstratified rimu seeds were germinated in a Jacobsen germinator at a 22°/18°C temperature regime; and unstratified seeds were germinated in soil in a glasshouse. Stratification had no effect on germination percent, however seeds germinated much more completely in the warm glasshouse.

Personal communications from D.J. Scott of the Ministry of Agriculture and Fisheries indicated that an average of 22 percent germination of rimu seed was achieved in a 30°C day (8 hours) and 20°C night (16 hours) regime. These few experiments give some indication of an appropriate temperature range for the germination of rimu seed.

It has been demonstrated for other species that optimum temperatures for germination may be expected to have a genetic component related to their climate of origin. A

germination experiment with rimu, kahikatea and totara would therefore be an important component of the investigation of the physiological and genetic differences between the three species. Unfortunately, totara seed was unavailable and this species could not be included in the experiment.

MATERIALS AND METHODS

Seed Origins

Nine hundred seeds of both rimu and kahikatea were taken from collections made by the New Zealand Forest Service. The rimu seed was collected from Hokitika. The kahikatea seed was collected from near Charleston (Appendix B). The seed had been stored moist at 4°C since collection.

Experimental Design and Conditions

Seed of both species was soaked in a 6250 ppm solution of Thiràm and rinsed once with distilled water. Seventy-five of the nine hundred seeds of each species were then soaked in a 500 ppm solution of gibberellic acid (GA_3) for 24 hours at 21°C, while the remaining seeds were kept moist at 21°C.

The seeds were then divided into lots of 25 and placed on two layers of Whatman No. 2 filter paper, moistened with distilled water, in plastic petri dishes. Each petri dish contained 75 seeds - three replicates of 25 seeds. The seeds soaked in gibberellic acid were kept separate from the others.

One petri dish of seed of each species was then placed in each of the ten compartments of a temperature gradient germinator in the Controlled Climate Laboratory of the Forest Research Centre, Christchurch (FRC). For each species, the seeds soaked in gibberellic acid and one petri dish of untreated seeds were placed in a diurnal temperature germinator, also at the FRC.

The extremes of the temperature gradient germinator were set at 7°C and 30°C and temperature varied between these extremes along the length of the copper base. The ten compartments of the germinator had temperatures of: $6.6^{\circ}\pm1.4^{\circ}C$, $10.5^{\circ}C\pm1.5^{\circ}C$, $13.0^{\circ}\pm1.0^{\circ}C$, $15.8^{\circ}\pm1.2^{\circ}C$, $18.3^{\circ}\pm0.7^{\circ}C$, $21.0^{\circ}\pm1.0^{\circ}C$, $22.0^{\circ}\pm0.5^{\circ}C$, $27.0^{\circ}\pm0.5^{\circ}C$ and $30.5^{\circ}\pm0.5^{\circ}C$. The compartments were constantly illuminated by warm white fluorescent lights with an intensity of 10 $\mu molm^{-2}s^{-1}$.

The diurnal temperature germinator operated on a 12 hour temperature cycle, alternating between 30° and 20°C. The germinator was lit constantly by warm white fluorescent tubes with an intensity of 30 $\mu molm^{-2}s^{-1}$.

The seeds were observed daily and kept moist with distilled water. When 2 mm of radicle had emerged from a seed, it was considered to have germinated and was recorded and removed from the petri dish.

When fungi or mould appeared on the seeds or filter paper, the filter paper was changed and the seeds washed in a 6250 ppm solution of Thiram, rinsed and replaced on the new filter paper.

The experiment ran for 175 days, and upon completion, all remaining seeds were dissected and stained with a 0.5 percent tetrazolium salt solution to test for viability.

Analysis

The mean percentage germination for each species and temperature was calculated as the total number of germinants in each of the three replicates divided by the total number of sound seed in each replicate (usually 25, as decided by the tetrazolium stain). These figures were then analyzed by species in an analysis of variance with a model as follows (see Appendix L for sums of squares):

SOURCE OF VARIATION	df
Temperature Treatment	11
Replicate	2
Error	22
Total	35

The means for each temperature were then compared using Scheffe's test with a confidence level of 0.95.

The values of the number of germinants with time for each species and temperature were transformed using a natural logarithm conversion. The values of time were also transformed in the same way and regressions calculated to estimate the rates of germination. The slopes of the regressions were compared by covariance analysis.

Finally, Djavanshir and Pourbeik's (1976) germination values were calculated for each species and temperature and plotted accordingly. The formula for germination value is as follows:

$$\text{Germination Value} = \frac{\Sigma \text{DGS}}{N} \times \text{GP} \times 10$$

where DGS = daily germination speed (cumulative % germination/number of days)
N = number of DGS's calculated during the test
GP = percent germination at the conclusion of the test.

RESULTS

For both kahikatea and rimu, the number of germinants was significantly ($p=0.01$) affected by temperature (Table 12). The optimum temperatures for germination, however, differed markedly.

The optimal temperature regime for the germination of rimu seed was a day/night regime of $30^{o}/20^{o}C$. Constant temperatures of $20.8^{o}C$ to $27^{o}C$ also produced good germination, but temperatures below $20^{o}C$ or above $30^{o}C$ were sub-optimal.

The maximum germination percentage for kahikatea was greater, and occurred over a broader and lower temperature

range than for rimu. Optimum temperatures for germination of this species were between 6.75° and 22.2°C. There was a significant decline in the number of germinants at temperatures of 25°C or more.

Table 12: Tests of significance of mean germination percentage [1]

Rimu		Kahikatea	
Chamber Temp °C	Mean Germination Percent	Chamber Temp °C	Mean Germination Percent
30.0/20.0	45.3	6.7	69.3
30.0/20.0 GA_3	37.3	18.3	69.3
25.0	36.0	13.3	66.6
27.0	33.3	10.7	65.3
22.2	33.3	16.3	65.3
20.8	28.0	20.8	62.7
30.5	13.3	22.2	57.3
18.3	12.0	25.0	38.7
16.3	8.0	30.0/20.0 GA_3	33.3
6.7	2.7	30.0/20.0	28.0
13.3	2.7	27.0	28.0
10.7	1.3	30.5	14.7

[1] Means enclosed by the same bar are not significantly different (p=0.05).

Treating the seeds with gibberellic acid had no significant effect on percentage germination for either species.

Table 13 shows that temperature not only affected the number of germinants, but also the rate of germination. The kahikatea seeds germinated about 20 days earlier than the rimu seeds. Their germination was generally more prolonged, however, so the germination rates are slightly lower than those of rimu.

While the slopes of the germination curves for rimu were not significantly different, maximum germination rates occurred at 20.8°C, 22.2°C and 30°/20°C. Although the highest germination rate was achieved at 20.8°C, this temperature

produced a low germination percentage. The start of germination was also significantly delayed at this temperature. The earliest germination occurred at the 30°/20°C and 25°C temperatures.

Table 13: Slopes of regressions of the natural log of the number of germinants against the natural log of time for those temperatures producing more than 5% germination

Chamber Temp °C	Rimu Germination Rate	Kahikatea Germination Rate
6.7		3.614
10.7		1.775
13.3		2.098
16.3	2.445	1.971
18.3	2.960	1.618
20.8	3.878	1.664
22.2	3.411	1.680
25.0	2.540	1.255
27.0	2.803	1.901
30.5	2.572	2.070
30.0/20.0	3.051	2.000
30.0/20.0 GA_3	2.276	2.662

The slopes of the germination curves for kahikatea were significantly different ($p=0.001$). Temperatures between 6.7° and 16.3°C and 27° and 30.5°C produced the most rapid germination in this species; however percentage germination was much lower at the warmer temperatures. The start of germination was also much delayed at 6.7°C.

Figure 6 plots Djavanshir and Pourbeik's (1976) Germination Value against temperature for both species. This value is reputed to take into account both germination speed and total germination percent, and to be suitable for comparisons between species. For the seedlots in this experiment, kahikatea had a much greater germinative energy than rimu at all temperatures. This greater germinative

energy was due to earlier germination and higher overall percentage germination in kahikatea. The curve of Germination Value peaks at a lower temperature for kahikatea than for rimu. This indicates an optimum germination temperature of approximately 18°C for kahikatea, and an alternating day/night regime of 30°/20°C for rimu.

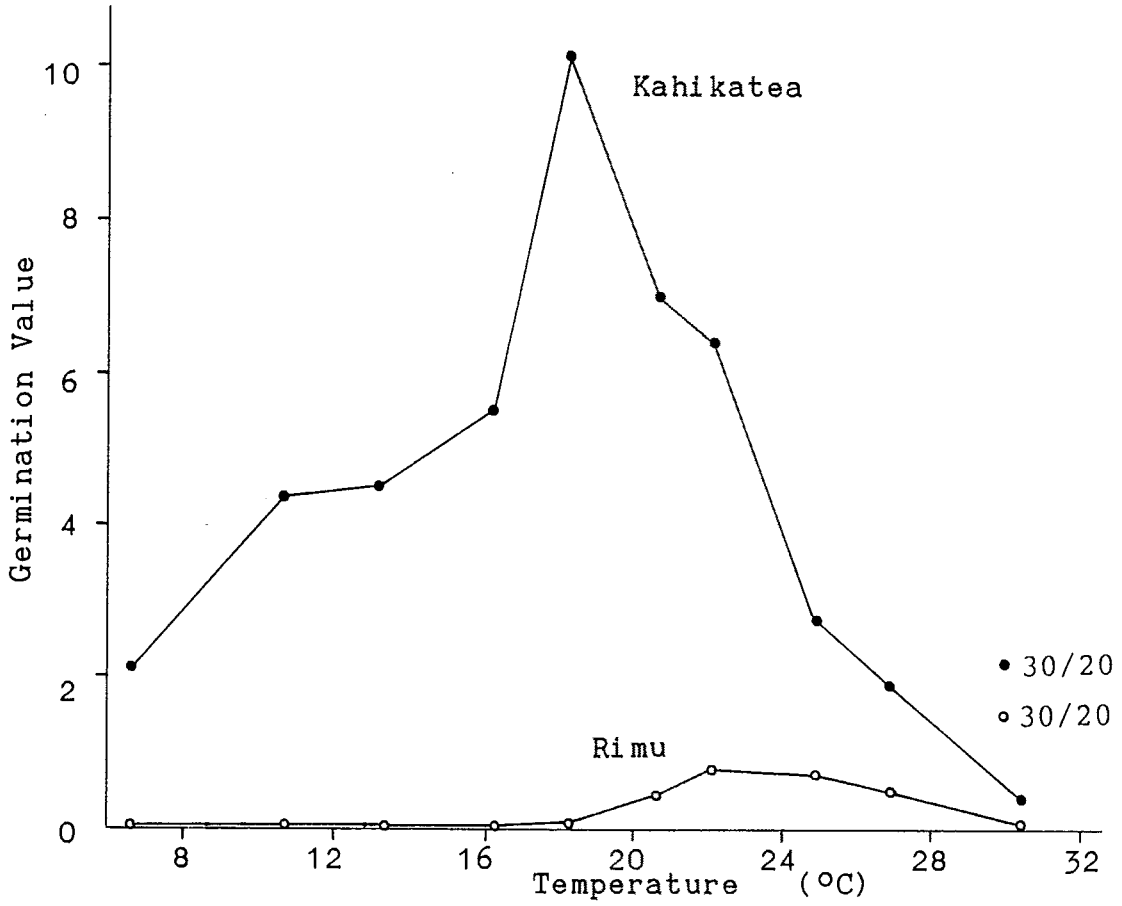

Figure 6: Plot of Germination Value (Djavanshir and Pourbeik 1976) versus temperature for two species

DISCUSSION

Before the results from this experiment are extended to seed of rimu and kahikatea in general, the experiment should be repeated with seed of different provenances. This was attempted in a second germination experiment, however

seed viability was so low that no conclusive results were obtained.

It does seem, however, that results from this experiment can be tentatively extended to rimu seed in general. In M.H. Bannister's 1955 trial, maximum percent germination of rimu seed was reached after 80 days in the germinator and 180 days in the soil. Bannister's germinator results compare well with the time of 60 to 70 days taken for rimu seed to germinate in the experiment under discussion.

The mean percent germination in Bannister's Jacobsen germinator was 13 percent, while it was 24.7 percent in the warm, glasshouse soil. It was suggested that the $22^{o}/18^{o}C$ regime in the germinator may have been too cool for optimum germination. This would correlate well with this experiment's results of 12 percent germination for rimu at a constant $18^{o}C$ temperature, and higher percentages at warmer temperatures.

D.J. Scott's figure of 22 percent germination at $30^{o}/20^{o}C$ mentioned in the Introduction is lower than the percent germination at those temperatures in this experiment probably because of Scott's extended, cool night temperatures.

It is interesting to note the effectiveness of the $30^{o}/20^{o}C$ regime on the germination of rimu seed. Toole et al. (1956) state that alternations from a temperature near the optimal constant value to some higher temperature which might be above the maximal is most effective in promoting germination. They suggest that the increased respiration and metabolism at the higher temperature changes the balance of the intermediate materials of the respiratory cycle. While this new balance might not be favourable for germination at continued high temperatures, it may promote germination with a change to a lower temperature. This is possibly why the alternating regime was optimal for the germination of rimu seed.

Other New Zealand species have been tested for optimum germination temperatures. Mohan et al. (1984) found temperature to significantly affect the germination rate, but not the final percentage germination, of manuka seed. As with rimu and kahikatea, lower temperatures delayed the onset of germination of manuka, with the earliest germination occurring at 25°C.

Mirams (1951) found the optimum temperature for kauri seed germination to be between 24° and 30°C; and Bieleski (1959) narrowed this to a range between 23° and 26°C. Barton (1978) further reduced this uncertainty and stated the best temperature for germination and initial growth in kauri was 25.5°C. He also found germination to be poor below 15.5°C or above 34°C. Rimu's range appears narrower than that for kauri, but the 25°C optima is very similar. Barton (1978) did not have a regime with alternating temperatures.

If the optimum temperature for germination is under genetic control, this similarity of rimu and kauri would seem a little curious, especially in light of the knowledge that the rimu seed was from the West Coast of the South Island. Climatic conditions could be expected to be somewhat cooler there than they would be in areas where kauri grows, and this response does not fit the theory that seed physiology is adapted to climatic conditions.

It also seems strange that the kahikatea seed which was collected from a location very near that of the rimu, had a temperature optima 7°C lower than that of the rimu. If the regenerative strategies of the two species were dissimilar, it could explain the difference; however this does not appear to be so. For instance, pioneer species in North America such as Douglas-fir, lodgepole pine and ponderosa pine seem to have much higher temperature requirements for germination (30°C) than do climax species such as western hemlock and western red cedar (20°C) (USDA, 1974). This might be an adaptation to the high soil-surface temperatures pioneer

species would be expected to encounter; whereas the climax species might be adapted to germinate in a cooler, closed canopy situation. This explanation, however, does not clarify the differences between rimu and kahikatea as both are most likely to regenerate under successional circumstances.

One inference that can be drawn from this trial is that current nursery practices for producing rimu seedlings do not maximize the seed's germinative potential. Doctrine dictates that seed should be sown in open ground beds with shade cloth covers, or in trays in an unheated glasshouse (T.J. Torrens, pers. comm.). Temperatures under these situations reach a maximum of 21^{o}C in January in Auckland, but generally are much cooler. According to the results detailed here, this is too cool for optimum germination; therefore if maximum return is desired from rimu seed collections, germination should be carried out under much warmer conditions.

Following on from these results, an investigation of optimum temperatures for seedling growth was undertaken to gain more information on the response of rimu, kahikatea and totara to temperature.

CHAPTER IV

THREE EXPERIMENTS TO DETERMINE THE EFFECT OF TEMPERATURE ON SEEDLING GROWTH

INTRODUCTION

The germination experiment produced some unexpected results, in that the optimum temperature for germination of rimu seed was so much above that which the seeds would experience under natural conditions. Following on from this, a controlled environment trial was designed to discover whether the high temperature optimum would extend to seedling growth of rimu, and in turn, how that would relate to the growth of kahikatea and totara.

Physiological Responses to Temperature

Temperature is one of the most significant influences on plant growth. Temperature controls plant growth and development by regulating the rate of all physical and biochemical processes within the plant (Downs and Hellmers, 1975). It influences photosynthesis, both directly and indirectly, by controlling respiration and transpiration (Kozlowski, 1971). Temperature determines the rate of diffusion of both organic and inorganic molecules within the aqueous system of plants, and it also affects the rates of biochemical reactions (Downs and Hellmers, 1975). These biochemical reactions are enzymatically controlled, and it is the effect of temperature on reaction rates of the enzyme systems which results in changes in the rates of growth processes.

Temperature optima for enzymatic reactions vary between enzyme systems. A change in temperature may increase the reaction rate of one enzyme, while decreasing the reaction rate of another. In addition, the process of

changing material from photosynthate to structural or storage products can often occur in more than one way; thus a change in temperature may merely cause a change to or from a more efficient process (Downs and Hellmers, 1975). It is these sensitive reactions that determine a temperature optimum for overall growth in a plant. Maximum growth is achieved when the rates of the majority of enzymatic processes are optimised and the processing of photosynthate is most efficient. At temperature extremes, the rate of growth may be limited by the rate of a single reaction (Downs and Hellmers, 1975).

Temperature requirements of a species can affect its range, its distribution within the range, and its growth rate (Hellmers, 1962). It is likely that selection pressures would act upon the temperature requirements of a plant species to fit it for survival and competition in the environments in which it grows. This would lead to varying temperature optima for species from different habitats. As Treshow (1970) states, "every plant species is best adapted to a particular temperature regime".

Temperature Optima of Other Tree Species

Much work has been done, particularly with conifers from the northern hemisphere, in determining optimum temperatures for growth. A wide variety of temperature controls have been found, with some species responding to day temperatures, others to night temperatures, and still others to overall heat sums. Some species require high day/night temperature differentials, while others prefer constant temperatures.

Douglas-fir seedlings have been studied fairly intensively, and this species has been shown to have a broad temperature optimum for growth between 18^{o} and $24^{o}C$. More northerly provenances appear to prefer the cooler temperatures of the range (Brix, 1971). Southerly

provenances would be subject to summer temperatures warmer than those in most of New Zealand. A constant day/night temperature has also been found to be as good as or better than an alternating regime for growth in this species (Brix, 1971).

Loblolly pine, a species from areas with high summer temperatures, requires at least a 12°C drop in night temperature, and grows best at 30°/17°C and 23°/11°C regimes (Kramer, 1957).

Engelmann spruce produces optimum growth under conditions where night temperatures are higher than day; and this species has optimum growth at a 19°/23°C regime (Hellmers et al., 1970).

Considering species from areas with climates more comparable to New Zealand, redwood seedlings were found to grow optimally in either a 19°/15°C or 19°/19°C temperature regime (Hellmers, 1966). Western hemlock seedlings have a pronounced temperature optimum of 18°C (Brix, 1971). Radiata pine, a species which has been proven to perform remarkably well in New Zealand's climate, produces maximum growth at day temperatures of 17° and 23°C and night temperatures of 5°C. The night temperature is considered to be the controlling influence on growth in this case (Hellmers and Rook, 1973). In that experiment, both Californian and New Zealand provenances were compared; and while they had the same optimum temperatures for growth, at higher temperatures, the Californian seedlings outperformed their New Zealand counterparts.

Trees from tropical and subtropical climates often show elevated temperature optima compared to those from more temperate climates. In Australia, Eucalyptus camaldulensis and Eucalyptus grandis, both species from hotter areas of that country, produce optimum growth at a 30°/25°C temperature regime (Sa-ardavut et al., 1984). This optimum is 5° to 9°C higher than that of other eucalypts from more

temperate regions. Temperature optima of tropical tree crops are discussed by Opeke (1982), and he suggests temperatures of 28°C for citrus trees, a range of 24° to 29°C for cashew, 24° to 35°C for rubber and 27° to 35°C for coconut and oil palms.

Another feature of trees from tropical environments is their preference for a uniform temperature regime. The high temperatures of the tropics are due largely to higher minima; and daily and seasonal differences can be as low as 1°C on tropical islands (Ewusie, 1980). Thus plants from these climates perform best at even temperatures.

It can be seen, even for these limited examples, that there is a wide range of conditions producing optimum growth in different species. Generally, these optimum conditions can be related to the native habitat of the species. Hellmers and Rook (1973) caution, however, that "although evolution can be expected to harmonize the growth mechanisms of a tree with its environment, the assumption should not be made that the native habitat of a species defines its potential environmental limits".

As temperature has such a profound effect on seedling growth and is therefore likely to be a strong selective pressure, it was considered a vital component in the comparison of the physiology of rimu, kahikatea and totara.

MATERIALS AND METHODS - FIRST TEMPERATURE EXPERIMENT

Seedling Origins

Eighty seedlings each of rimu, kahikatea and totara were potted into 0.25 l plastic pots six weeks prior to the beginning of the experiment. The seedlings used in this experiment were from seed collected by the New Zealand Forest Service in 1985. The rimu seed was from Hokitika, the kahikatea seed from Charleston and the totara seed from Te Karaka (Appendix B).

The rimu and kahikatea seedlings were germinated as part of the germination experiment, and then were grown for several months in the glasshouse prior to potting. The totara seedlings had been germinated in soil and grown in the glasshouse for two months before being potted.

The potting mix was made up according to the MAF (1985) Aglink publication HPP 138, and contained: 40 percent sand, 30 percent ground bark and 30 percent peat, with 4 kg dolomite lime, 1 kg superphosphate, 1 kg calcium ammonium nitrate, 4 kg Osmocote, and 150 g trace element mix per cubic metre of soil.

Controlled Environment Cabinet Conditions

On Sept. 2, 1986, twenty seedlings of each species were transferred to each of three Controlled Environment (C.E.) cabinets (Temperzone, Auckland) in the Controlled Climate Laboratory of the Forest Research Centre, Christchurch. Twenty seedlings of each species were also left in the glasshouse.

The C.E. cabinets were set for a 16 hour photoperiod and a relative humidity of 70 percent. Each C.E. cabinet had a 5°C day/night temperature differential corresponding to the daylight regime. The cabinet temperature regimes were: 27°/22°C, 21°/16°C and 15°/10°C. These temperatures were chosen on the basis of a literature review of trials with other species, and the germination experiment. Temperature and humidity in the glasshouse were monitored. Temperature averaged 18°C for 16 hours and 27°C for 6 hours, with the changes spanning one hour. Relative humidity averaged 85 percent for 16 hours and 65 percent for 6 hours, and the plants were subject to natural photoperiods.

After five days in the C.E. cabinets, many of the seedlings were taking on a reddish coloration and showing signs of stress. It was decided that the light regime in the C.E. cabinets was too intense and this was accordingly

reduced to 10 hours full light with 3 hours of only incandescent lights before and after the 10 hours of full light. Shade cloth which reduced the light by approximately 30 percent was also suspended over the seedlings, and these measures alleviated the stress symptoms. The final average daytime light levels in the three C.E. cabinets were: $27^{o}/22^{o}C$ - 450 $\mu molm^{-2}s^{-1}$, $21^{o}/16^{o}C$ - 400 $\mu molm^{-2}s^{-1}$, $15^{o}/10^{o}C$ - 440 $\mu molm^{-2}s^{-1}$, glasshouse (bright day) - 550 $\mu molm^{-2}s^{-1}$.

The seedlings were watered on a pot weight basis. Each C.E. cabinet had sample seedlings of a known pot weight, and when these samples lost 10 g of water, all pots were given 10 ml of distilled water. Excess water was allowed to drain from the pots.

Blocking By Size

When seedlings of each species were divided among the temperature regimes, the four tallest trees were randomly allocated to one of the treatments. The next four tallest trees were again randomly allocated, and so on until each treatment contained twenty seedlings of each species. The twenty seedlings were then split into five blocks of four seedlings in order of decreasing height. This systematic distribution was done so the effect of initial size could be statistically removed at the time of analysis. The seedlings of all species were then randomly arranged within the C.E. cabinets and on the glasshouse bench, and were re-randomized every two weeks to account for variation in microclimates.

Measurements of Growth

The experiment lasted 68 days at which time the seedlings were removed to cold storage until they could be analyzed. They had been measured for height and number of branches at the start of the trial and every month thereafter; and this measurement was taken again. The number of leaves per tree was counted and each seedling was divided

into leaf, stem and root portions. These portions were combined for each four-seedling block, and the combined tissues were oven-dried at 80°C for 48 hours and then weighed.

Analysis

The measured growth variables of total weight, leaf weight, stem weight, root weight, height, number of branches and number of leaves, and the ratios of leaf, stem and root weight to total weight for all three species were compared using an analysis of variance. As there were unequal numbers of observations, the General Linear Model Procedure (SAS, 1985) was used. The model was as follows (see Appendix M for sums of squares):

SOURCE OF VARIATION	df
Species	2
Temperature Treatment	3
Size Class	4
Species X Size Class	8
Error	42
Total	59

The 'Error' term was comprised of the following terms:

SOURCE OF VARIATION	df
Treatment X Species	6
Treatment X Size Class	12
Treatment X Species X Size Class	24

As the amount of variation explained by these interactions was not significant, the terms were pooled to increase precision.

The means of each species, temperature, and species X temperature combination were compared using Scheffe's test with a confidence level of 0.95.

RESULTS OF THE FIRST TEMPERATURE EXPERIMENT

Results from this trial indicated that rimu, kahikatea and totara produced maximum height growth and dry weight at a 27°/22°C temperature regime (Plate 7).

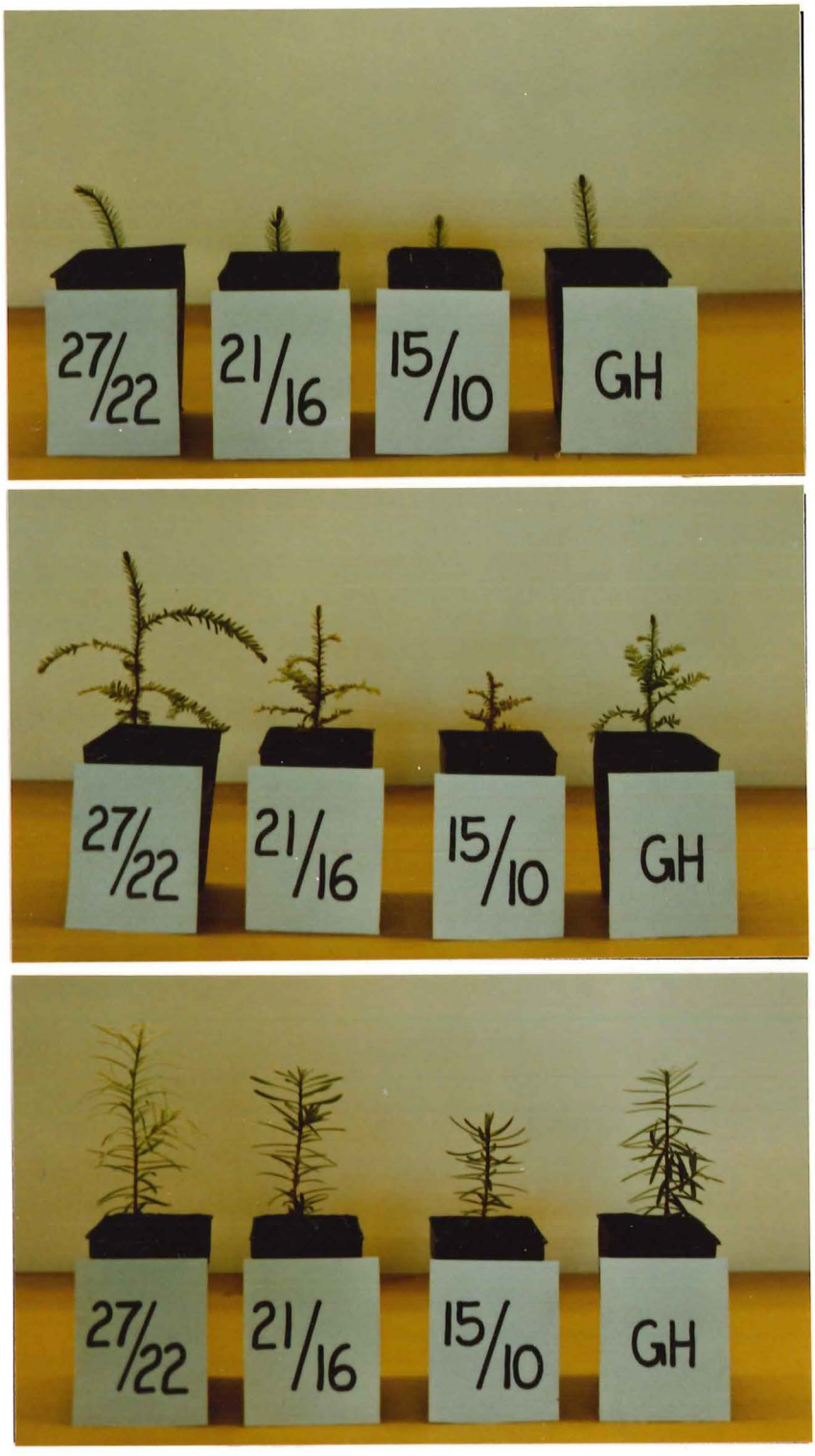

Plate 7: Average seedlings of rimu (top), kahikatea (centre) and totara (bottom) from the first temperature experiment

Obvious species differences were exhibited (Table 14). Generally, totara seedlings were significantly larger in all respects than seedlings of the other two species. The exceptions to this were for stem weight and root weight. Kahikatea appeared to put a significantly higher proportion of assimilate into stem tissue than the other species, while rimu put a significantly higher proportion into root tissue.

All measured growth variables except root weight/total weight showed increased growth at increased temperatures. Significance was not always clearly demarcated. All species allocated a significantly higher proportion of resources to root tissue at low temperatures.

If the species were independently analyzed for the same variables, the trend of increased growth at higher temperatures was again clear; but different species expressed significance for different variables (Table 14).

In terms of total weight, totara appeared to have the greatest response to an increase in temperature, with a 17.3 percent increase in dry weight between the 21°C and 27°C regimes. Kahikatea and rimu had more modest increases of 5.6 and 4.6 percent respectively. Looking at leaf weight as a proportion of total weight however, gives a different perspective. Rimu had the greatest response in leaf production, putting 12.2 percent more growth into leaves in the warmest cabinet. Totara and kahikatea had a slight decrease in the allocation of resources to leaves between the 21° and 27°C regimes.

Trees in the glasshouse generally exhibited behaviour similar to those in the 21°/16°C cabinet. Values for the variables were usually not significantly different from that C.E. cabinet, but were commonly slightly lower.

Table 14: Tests of significance for measured variables in the first temperature experiment [1]

Rimu Temp °C	Mean	Kahikatea Temp °C	Mean	Totara Temp °C	Mean
TOTAL WEIGHT (g)					
Totara > Kahikatea > Rimu[2]					
27/22	0.181	27/22	0.748	27/22	1.712
21/16	0.173	21/16	0.708	21/16	1.460
15/10	0.123	15/10	0.460	15/10	1.069
LEAF WEIGHT/TOTAL WEIGHT					
Totara > Rimu > Kahikatea[2]					
27/22	0.468	21/16	0.425	15/10	0.613
21/16	0.417	27/22	0.422	21/16	0.596
15/10	0.403	15/10	0.394	27/22	0.595
STEM WEIGHT/TOTAL WEIGHT					
Kahikatea > Rimu > Totara[2]					
27/22	0.199	27/22	0.270	27/22	0.166
21/16	0.176	21/16	0.260	21/16	0.127
15/10	0.161	15/10	0.226	15/10	0.103
ROOT WEIGHT/TOTAL WEIGHT					
Rimu > Kahikatea > Totara[2]					
15/10	0.436	15/10	0.373	15/10	0.284
21/16	0.406	21/16	0.315	21/16	0.262
27/22	0.333	27/22	0.308	27/22	0.239
HEIGHT (mm)					
Totara > Kahikatea > Rimu[2]					
27/22	52.1	27/22	80.1	27/22	102.3
21/16	38.1	21/16	73.2	21/16	80.1
15/10	29.7	15/10	42.7	15/10	51.3
NUMBER OF LEAVES[3]					
Kahikatea > Rimu > Totara[2]					
27/22	700.4	27/22	2020.4	27/22	630.8
21/16	459.2	21/16	1759.4	21/16	312.8
15/10	322.4	15/10	1077.0	15/10	191.8

[1] Values spanned by the same bar are not significantly different (p=0.05).

[2] Species differences significant at the p=0.01 level

[3] Number of leaves was well correlated with leaf weight for rimu and kahikatea (R^2= 0.79 and 0.85 respectively). For totara, the correlation was poor (R^2= 0.54).

When the natural log of height growth of the size classes of each species was plotted over time, the lines were basically parallel (Figure 7). This demonstrates that, for these species, blocking by size class is a viable option to reduce variation in the analysis.

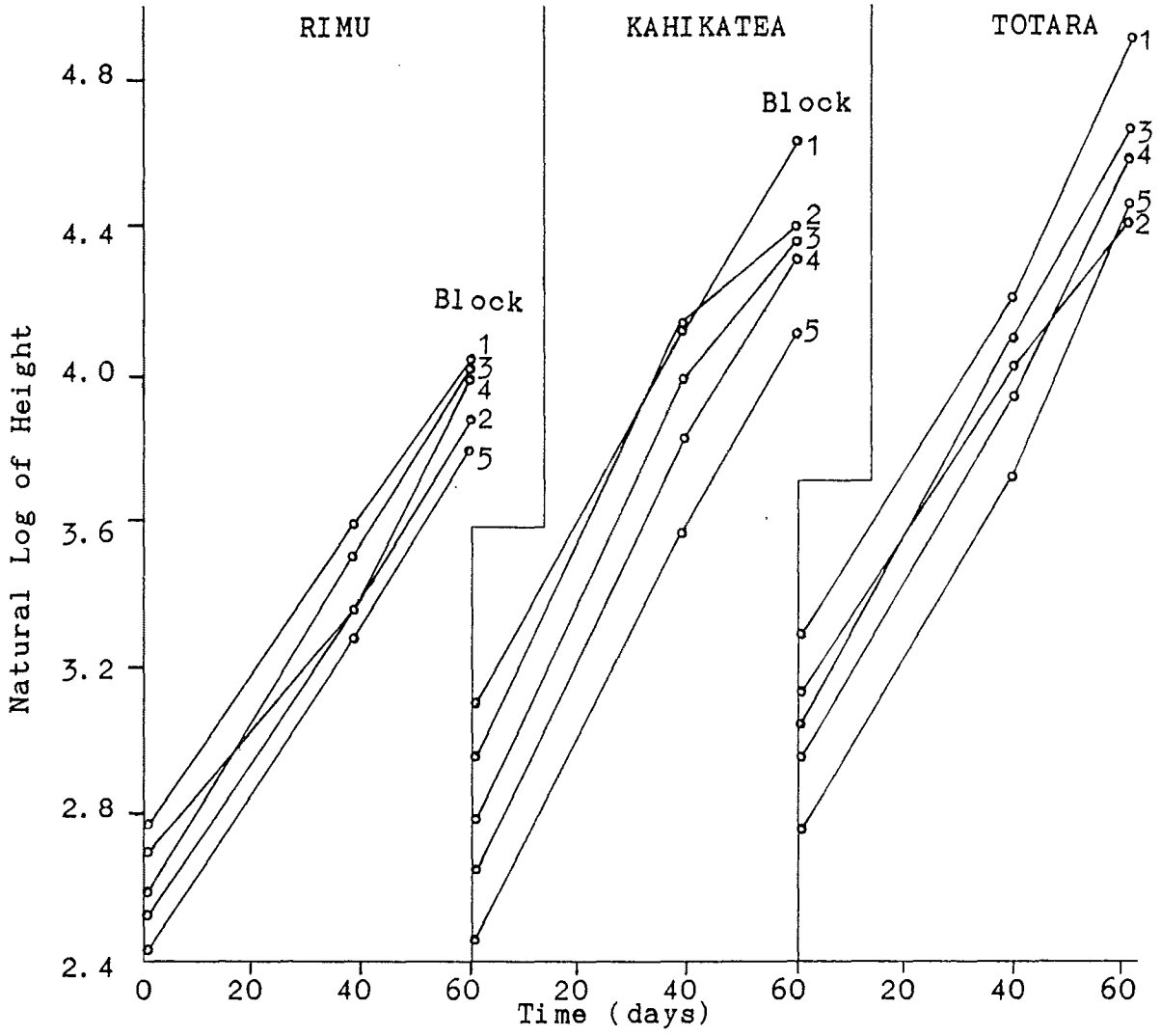

Figure 7: Plot of average height growth versus time for blocks of five seedlings in the 27°/22°C C.E. cabinet

INTRODUCTION TO THE SECOND TEMPERATURE EXPERIMENT

In the first temperature experiment, it was discovered that rimu, kahikatea and totara produced their greatest growth in a 27°C temperature regime. As in the germination experiment, the optimum temperature was much higher than would be expected given present climatic conditions in New Zealand.

As a deleterious temperature regime had not yet been reached, a second temperature experiment was needed to discover the upper limit of the three species' response to temperature. The first temperature experiment also raised questions about the seedling's response to light intensity, and this warranted further investigation.

A second temperature experiment offered the opportunity to explore the comparative responses of New Zealand species from warmer or cooler ecotypes, so seedlings of kauri and mountain beech were included. A constant temperature regime was also incorporated as other species have been shown to grow optimally under such conditions.

The second temperature experiment then, was designed to resolve questions raised by the first experiment, and to further explore the significance of these results.

MATERIALS AND METHODS - SECOND TEMPERATURE EXPERIMENT

Seedling Origins

Seventy-five seedlings each of rimu, kahikatea, totara, mountain beech and kauri were potted into 0.6 l plastic pots. The rimu, kahikatea and totara had been germinated four to five months prior to this time, and had been growing in the glasshouse. These species were grown from seed of the same collections as were used in the first temperature experiment. The kauri seedlings had been germinated five months prior and were growing indoors up to

this time. The kauri seed was collected from six sites near Whangarei in Northland (Rouse, 1986). The beech were two to five years old, and were collected in October, 1986 from a gravelly stream bank at the headwaters of the Broken River, at approximately 1000 m elevation (G. Baker, pers. comm.). They were brought directly to the university. All species, once potted, were kept in a uniform environment in the glasshouse for at least four weeks before the experiment began.

The potting mix used was very similar to that in the first temperature experiment, however it was a commercial mix - Odering's Nine Month Potting Mix - diluted with seven parts sand and two parts peat to nine parts potting mix.

Controlled Environment Cabinet Conditions

On Nov. 27, 1986, fifteen seedlings of each species were transferred to each of five C.E. cabinets (Temperzone, Auckland); three in the Controlled Climate Laboratory of the Forest Research Centre, Christchurch, and two in the glasshouse complex of the Department of Plant and Microbial Sciences, University of Canterbury. The C.E. cabinets had previously been divided into three light regimes - no shade, 30 percent shade, and 50 percent shade - and wooden frames covered in the appropriate weight of shade cloth had been placed in the C.E. cabinets. Five seedlings of each species were randomly arranged in each light regime.

The seedlings of each species had been divided into five size classes by estimated total weight. As in the first temperature experiment, the fifteen seedlings of each species in the largest size class were distributed randomly among each light regime and C.E. cabinet, then the next largest fifteen seedlings were similarly distributed and so on. This was done in order to minimize the effects of initial seedling size on the final analysis. The seedlings within each light regime were re-randomized every two weeks to account for

possible microclimate variation within the C.E. cabinets.

The C.E. cabinets were again set for a 16 hour photoperiod with three hours of incandescent lights before and after the ten hours of full light. The actual temperature, relative humidity and light regimes were as in Table 15. The relative humidities were chosen to produce a maximum vapour pressure deficit of 12 mb.

The seedlings were watered on a pot weight basis. Sample seedlings of a known pot weight were in each C.E. cabinet, and when these samples lost 15 g of water, all pots were given 15 ml of distilled water. Excess water was allowed to drain from the pots.

Table 15: Temperature, relative humidity and light regimes of the five C.E. cabinets

Temperature Regime	R.H. %	Light Regime ($\mu molm^{-2}s^{-1}$) No shade	30% shade	50% shade
35°/30°C	80	510	335	240
31°/26°C	76	660	410	300
27°/27°C	68	500	325	240
27°/22°C	68	495	340	260
21°/16°C	55	650	420	300

Measurement of Photosynthesis

Ninety-five days into the experiment, four trees of each species were selected from each C.E. cabinet for measurements of photosynthetic rates. Generally these trees were taken from the 'no shade' light regime, but if four healthy trees were not available, the remainder were taken from the '30 percent shade' regime. A LI-6000 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska) incorporating a 1 l capacity cuvette was used to measure photosynthetic rates. Two measurements were taken per tree. Anything from a branch to an entire tree was used to obtain

the readings in order to get a 30 ppm drawdown in CO_2 level over the 90 s measurement period. Attempts were made to achieve uniform light levels for all measurements in all cabinets, and cuvette air conditions were maintained similar to those of the C.E. cabinet. Net photosynthetic rates were calculated on a total leaf surface area basis. Leaf area was measured with a Delta-T Area Meter.

Measurement of Growth

The experiment lasted 100 days, at which time the trees were removed by species to cold storage. Each species was measured and dissected before the next species was removed from the C.E. cabinets.

At the beginning of the experiment, the rimu, kahikatea, totara and kauri seedlings had been measured for height and branch number, and these measurements were taken again. Height was measured from the cotyledons to the tip of the leaves highest above the soil for kauri. All species were measured for stem diameter at soil level and leaf area. Leaf area was measured with a Delta-T Area Meter. The dissected leaf, stem and root tissues were oven-dried at 80°C for 48 hours and then weighed.

Analysis

The measured growth variables of total weight, root, stem and leaf weight, height, stem diameter and leaf area, and the ratios of the portion weights to total weight were compared by analysis of variance separately for each species. Values from the 35°/30°C temperature regime were not included as seedling mortality was so high. As there were unequal numbers of observations, the General Linear Models Procedure (SAS, 1985) was used. The model was for a split-plot analysis and was as follows (assuming no missing data) (see Appendix N for sums of squares):

	SOURCE OF VARIATION	df
Whole Plot	Temperature Treatment	3
	Light Treatment	2
	Error A	6
Split-Plot	Size Class	4
	Size Class X Light	8
	Error B	36
	Total	59

The 'Error B' term was comprised of the following elements:

SOURCE OF VARIATION	df
Temperature X Size Class	12
Temperature X Light X Size Class	24

These interactions were not significant, and so their variation was pooled to increase precision.

The means of the variables at different temperatures were compared using Scheffe's test.

The photosynthetic rates of the individual species at the different temperatures were also compared by analysis of variance, and the means tested using Scheffe's test. The model was as follows (see Appendix O) for sums of squares):

SOURCE OF VARIATION	df
Temperature Treatment	3
Error	28
Total	31

The 'Error' term was comprised of the following elements:

SOURCE OF VARIATION	df
Between Trees	3
Between Measurements	1
Treatment X Trees	9
Treatment X Measurements	3
Trees X Measurement	3
Treatment X Trees X Measurement	9

As none of these interaction terms were significant, they were pooled to form one error term to increase precision.

For total weight, the ratio of leaf weight to total weight, and photosynthetic rate, the percentage changes in values from the $21^{o}/16^{o}C$ regime to the $27^{o}/22^{o}C$ regime, and the $27^{o}/22^{o}C$ regime to the $31^{o}/26^{o}C$ regime were calculated.

This was the absolute difference of the variable values at the two temperatures, divided by the value at the lower temperature, multiplied by 100.

The initial and final heights of rimu, kahikatea and totara at each temperature were transformed by taking their natural log, and these values were plotted against time. These transformation were done in order to change the growth curves to straight lines.

RESULTS OF THE SECOND TEMPERATURE EXPERIMENT

Growth

Results from this second temperature experiment are presented in Tables 16, 17, 18 and 19; and Figures 8, 9 and 10. The warmest temperatures in the 35°/30°C regime were highly detrimental to the growth of all species, and these results were not included in the tables.

Table 16 summarizes the main aspects of Tables 17, 18 and 19, and provides an insight into the response mechanisms of the five species. This table presents the percentage changes in total weight, leaf weight/total weight and photosynthesis for all species as temperatures rose from 21°/16°C to 27°/22°C, and from 27°/22°C to 31°/26°C.

All species increased in total weight between 21°C and 27°C; however rimu and totara were the most responsive to this increase in temperature. Kauri was the least responsive. All species decreased in total weight between 27°C and 31°C, but at these temperatures, beech had the most negative response.

These trends were also displayed for the other measured growth variables (Table 17 and Figures 8, 9 and 10). Most species had maximum growth at 27°/22°C and minimum growth at 31°/26°C. The exception to this was the root growth of kauri and beech. These species exhibited an inverse relationship of root weight with temperature.

Table 16: The increase in total weight, leaf weight ratio and rate of net photosynthesis with changing temperature as a percentage of their values at the lower temperature in the second temperature experiment

	Rimu	Kahikatea	Totara	Kauri	Beech
TOTAL WEIGHT					
21/16 to 27/22	41.7	27.0	40.0	8.3	22.8
27/22 to 31/26	-64.7	-66.0	-45.4	-57.7	-73.3
LEAF WEIGHT/TOTAL WEIGHT					
21/16 to 27/22	30.4	-6.4	6.0	4.7	38.9
27/22 to 31/26	-7.0	2.6	-5.4	-7.1	-58.3
PHOTOSYNTHESIS					
21/16 to 27/22	2.4	5.2	43.6	25.9	-29.0
27/22 to 31/26	-57.8	-34.6	-66.7	-42.6	-65.6

While totara exhibited a similar pattern of growth to the other species, it showed no significant differences in growth between the various temperatures. Seedling size was highly variable, and this contributed to the lack of significance. If the three largest and three smallest observations were deleted, decreasing the variation, the growth in the 27°C regimes could be shown to be significantly ($p=0.05$) greater than in cooler or warmer regimes.

Table 17: Tests of significance between temperatures for measured variables in the second experiment [1]

Rimu		Kahikatea		Totara		Kauri		Beech	
Temp °C	Mean	Temp °C	Mean	Temp °C	Mean	Temp °C	Mean	Temp °C	Mean
TOTAL WEIGHT (g)									
27/22	0.17	27/27	0.96	27/22	1.54	27/22	0.26	27/22	2.21
27/27	0.16	27/22	0.94	27/27	1.31	21/16	0.24	21/16	1.80
21/16	0.12	21/16	0.74	21/16	1.10	27/27	0.20	27/27	1.34
31/26	0.06	31/26	0.32	31/26	0.84	31/26	0.11	31/26	0.59
LEAF WEIGHT (g)									
27/22	0.08	27/27	0.45	27/22	0.78	27/22	0.13	27/22	0.93
27/27	0.07	27/22	0.38	27/27	0.67	21/16	0.11	27/27	0.60
21/16	0.04	21/16	0.31	21/16	0.53	27/27	0.10	21/16	0.57
31/26	0.03	31/26	0.13	31/26	0.41	31/26	0.05	31/26	0.17
STEM WEIGHT (g)									
27/22	0.04	27/22	0.33	27/22	0.46	27/22	0.09	27/22	0.96
27/27	0.04	27/27	0.29	27/27	0.40	21/16	0.08	21/16	0.90
21/16	0.03	21/16	0.21	21/16	0.37	27/27	0.06	27/27	0.52
31/26	0.02	31/26	0.10	31/26	0.29	31/26	0.04	31/26	0.27
ROOT WEIGHT (g)									
27/22	0.05	27/22	0.23	27/22	0.30	21/16	0.05	21/16	0.33
21/16	0.05	27/27	0.22	27/27	0.24	27/22	0.04	27/22	0.32
27/27	0.04	21/16	0.21	21/16	0.20	27/27	0.03	27/27	0.22
31/26	0.02	31/26	0.09	31/26	0.14	31/26	0.02	31/26	0.15
LEAF AREA (cm^2)									
27/22	11.5	27/22	85.0	27/22	71.3	27/22	17.2	27/22	133
27/27	11.2	27/27	79.4	27/27	67.8	27/27	14.4	27/27	101
21/16	5.7	21/16	52.9	21/16	37.4	21/16	13.9	21/16	63
31/26	4.4	31/26	21.8	31/26	33.7	31/26	6.4	31/26	28
HEIGHT (mm)									
27/27	91	27/22	193	27/22	205	27/22	69		
27/22	82	27/27	174	27/27	189	27/27	63		
21/16	60	21/16	140	31/26	173	21/16	61		
31/26	54	31/26	95	21/16	169	31/26	38		
DIAMETER (mm)									
21/16	1.41	27/22	2.84	27/22	2.43	27/22	1.77	27/22	3.03
27/22	1.36	27/27	2.76	21/16	2.30	21/16	1.68	21/16	3.01
27/27	1.28	21/16	2.65	27/27	2.20	27/27	1.53	27/27	2.57
31/26	0.92	31/26	1.93	31/26	2.07	31/26	1.38	31/26	2.27

[1] Values spanned by the same bar are not significantly different (p=0.05).

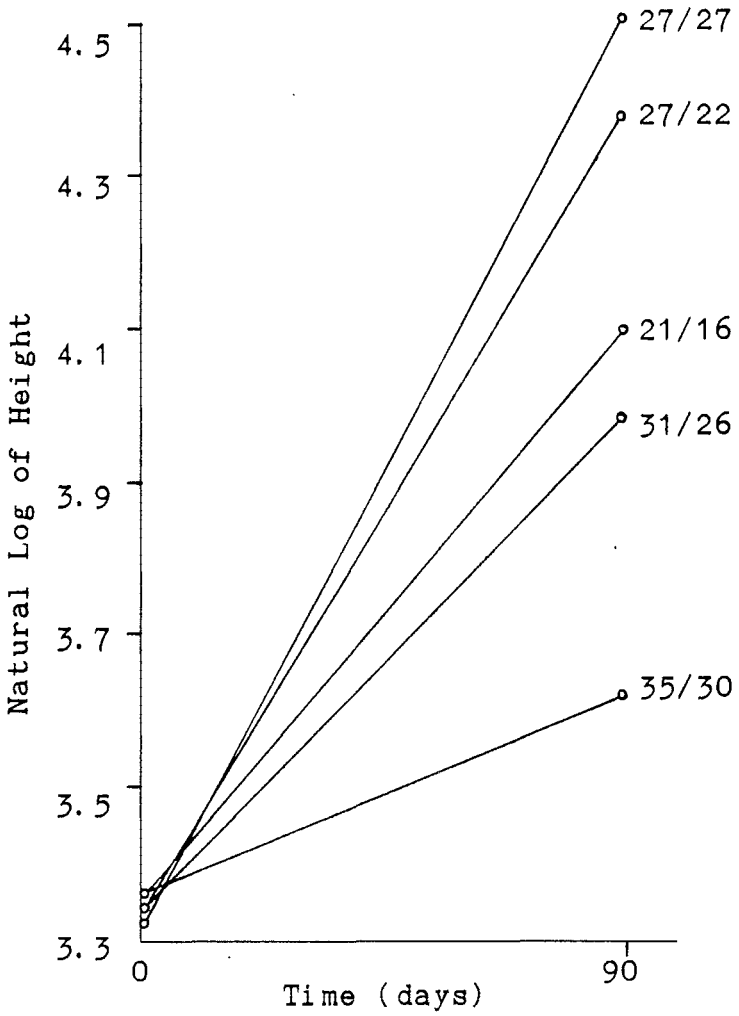

Figure 8: Plot of the natural log of height of rimu versus time for five temperature regimes

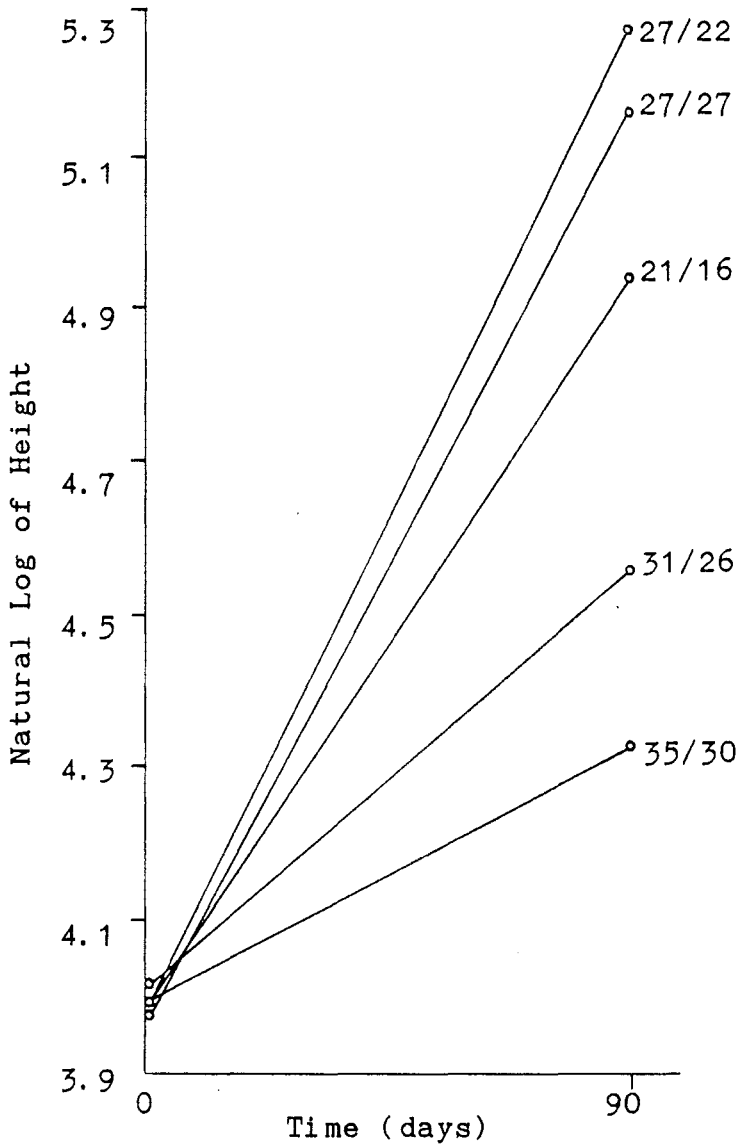

Figure 9: Plot of the natural log of height of kahikatea versus time for five temperature regimes

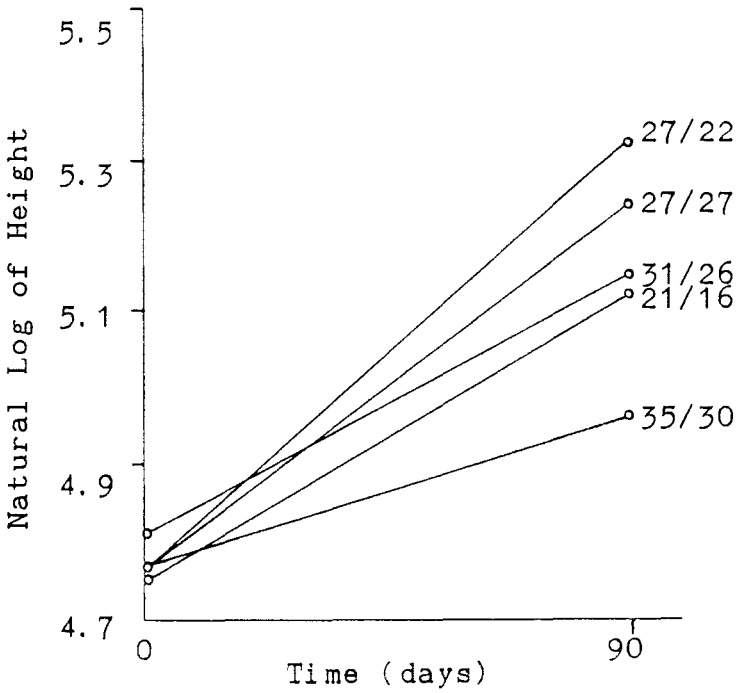

Figure 10: Plot of the natural log of height of totara versus time for five temperature regimes

Allocation of Resources and Net Photosynthesis

The percentage increases in total weight between the 21°C and 27°C regimes were associated with varying leaf weight and photosynthetic responses in the five species (Table 16). Increasing total weight of rimu and beech was associated with a major increase in leaf growth. This can also be seen in Table 17 which shows the leaf weight and leaf area of those species doubling between 21°C and 27°C.

Rimu and beech not only showed the greatest leaf weight response, they also appeared to have a different response in leaf structure from the other species. Table 18 presents the ratio of leaf area to leaf weight, and it can be seen that rimu and beech produced relatively thinner leaves at the highest temperatures.

Table 18: Tests of significance of allocation of dry weight to leaves, stems and roots in the second temperature experiment [1]

Rimu		Kahikatea		Totara		Kauri		Beech	
Temp °C	Mean	Temp °C	Mean	Temp °C	Mean	Temp °C	Mean	Temp °C	Mean
LEAF WEIGHT/TOTAL WEIGHT									
27/27	0.48	27/27	0.46	27/22	0.51	27/27	0.51	27/27	0.42
27/22	0.47	21/16	0.41	27/27	0.50	27/22	0.49	27/22	0.42
31/26	0.44	31/26	0.39	31/26	0.48	21/16	0.47	21/16	0.30
21/16	0.36	27/22	0.38	21/16	0.48	31/26	0.45	31/26	0.27
STEM WEIGHT/TOTAL WEIGHT									
31/26	0.29	27/22	0.36	31/26	0.34	31/26	0.37	21/16	0.48
27/22	0.24	31/26	0.33	21/16	0.33	27/22	0.34	31/26	0.46
27/27	0.24	27/27	0.30	27/27	0.30	21/16	0.33	27/22	0.42
21/16	0.23	21/16	0.29	27/22	0.28	27/27	0.33	27/27	0.40
ROOT WEIGHT/TOTAL WEIGHT									
21/16	0.41	21/16	0.31	27/22	0.20	21/16	0.20	31/26	0.27
27/22	0.29	31/26	0.28	27/27	0.19	31/26	0.17	21/16	0.21
31/26	0.28	27/22	0.26	21/16	0.18	27/22	0.17	27/27	0.18
27/27	0.27	27/27	0.24	31/26	0.17	27/27	0.16	27/22	0.16
LEAF AREA/LEAF WEIGHT (cm^2/g)									
21/16	130	21/16	169	21/16	71	21/16	121	21/16	111
27/22	139	31/26	169	31/26	83	31/26	128	27/22	143
27/27	148	27/27	176	27/22	91	27/22	133	31/26	158
31/26	158	27/22	221	27/27	101	27/27	140	27/27	168

[1] Values spanned by the same bar are not significantly different (p=0.05).

Table 18 also shows that kauri and totara had the greatest overall leaf production, while beech put the greatest proportion of dry matter into stem tissue. Rimu again had the greatest allocation of assimilate to root production.

For kauri, totara and, to a lesser extent, kahikatea, an increase in total weight between 21°C and 27°C was associated with an increase in photosynthetic rate (Table 19).

Table 19: Test of significance of mean net photosynthetic rates in the second temperature experiment [1]

Rimu		Kahikatea		Totara	
Temp °C	Photosynthesis $mgCO_2/m^2s$	Temp °C	Photosynthesis $mgCO_2/m^2s$	Temp °C	Photosynthesis $mgCO_2/m^2s$
27/22	0.128	27/27	0.099	27/27	0.151
21/16	0.125	27/22	0.081	27/22	0.135
27/27	0.083	21/16	0.077	21/16	0.094
31/26	0.054	31/26	0.053	31/26	0.045

Kauri		Mountain Beech	
Temp °C	Photosynthesis $mgCO_2/m^2s$	Temp °C	Photosynthesis $mgCO_2/m^2s$
27/22	0.141	21/16	0.303
21/16	0.112	27/22	0.215
27/27	0.092	27/27	0.147
31/26	0.081	31/26	0.074

[1] Values spanned by the same bar are not significantly different (p=0.05).

Kahikatea was the least responsive in terms of photosynthesis having the narrowest spread of photosynthetic rates. Beech was unique in showing a trend of decreasing photosynthetic rate with increasing temperature. Due to differences in leaf morphology and placement of stomata, photosynthetic rates of the five species are not comparable.

Beech was the most responsive species to a change in temperature from 27°C to 31°C (Table 16). For all species, the major decline in total weight between these temperatures was associated with a reduction in net photosynthesis. In beech this was accompanied by a major decrease in leaf production. The decrease in the net photosynthesis of all species in the warmest temperatures was presumably caused by an increase in respiration rate.

The Effect of Light Intensity

In general, the various light levels made no significant difference to growth in any species. Kauri did show a significant ($p=0.05$) interaction between light and temperature for total weight, with better growth at lower light intensities in warmer temperatures; but this did not extend to other growth variables. Although it was not significant, all measurements of totara's growth were minimum under full light.

This same trend was exhibited in mortality figures in the $35^{o}/30^{o}C$ temperature regime. At these high temperatures, mortality levels for totara, kauri and beech declined with light intensity.

DISCUSSION OF THE EFFECTS OF TEMPERATURE AND LIGHT ON SEEDLING GROWTH - FIRST AND SECOND TEMPERATURE EXPERIMENTS

Response to Light

Light intensity affects the growth of shoots through its effects on photosynthesis, stomatal opening, and chlorophyll and hormone synthesis (Kozlowski, 1971). Species vary greatly in their response to light and ability to withstand low light intensities (Kozlowski, 1971); and rimu, at least, is known to be highly tolerant of low light levels (McEwen, 1983).

Warrington et al. (1989) found that light intensities of 600 $\mu molm^{-2}s^{-1}$, as compared to 200 $\mu molm^{-2}s^{-1}$, increased height and dry weight growth in rimu and kauri. These differences, however, did not become apparent until 150 days into their trial. These authors found rimu to have a much weaker response to light intensity than the more shade intolerant kauri, however the patterns of allocation of new growth were similar in the two species, with increased allocation going to foliage and stem.

In the experiment under discussion, light intensity made no significant difference to growth of the five species, and this may be because the range of light intensities was too narrow, or the duration of the experiment too short. Introducing the two shade screens to the cabinets reduced the intensity in the 100 percent light treatment, and this may explain why the adverse reaction to full light obtained in the first temperature experiment did not occur in the second.

Responses to Temperature

For all species under study, growth was maximized in the 27°C regimes in both temperature experiments. While growth at 27°C was not always significantly different from that at 21°C, the repeated occurrence of a 27°C optimum gives weight to this conclusion (Plate 8).

In both temperature experiments, rimu allocated a higher percentage assimilate to leaf production at 27°C. Beech also followed this trend, while other species merely increased their photosynthetic rates at the higher temperatures. From the figures in Table 18, it seems that rimu and beech are more able to continue increasing leaf surface area as temperatures increase. Beech may be able to dissipate rising internal heat through increased transpiration, while rimu may do this through effective convection around its small leaves; thus these two species may be more likely to continue putting resources into leaf production as temperatures rise.

The trend of decreasing root weight with increasing temperatures seen in kauri, beech and probably rimu, kahikatea and totara, is common to other species. Scots pine, European larch, pitch pine (Gowin et al., 1980; Good and Good, 1976), and radiata pine (Hellmers and Rook, 1973) all showed decreasing root biomass with increasing temperature. This may indicate that root meristems have different and lower temperature optima from shoot meristems.

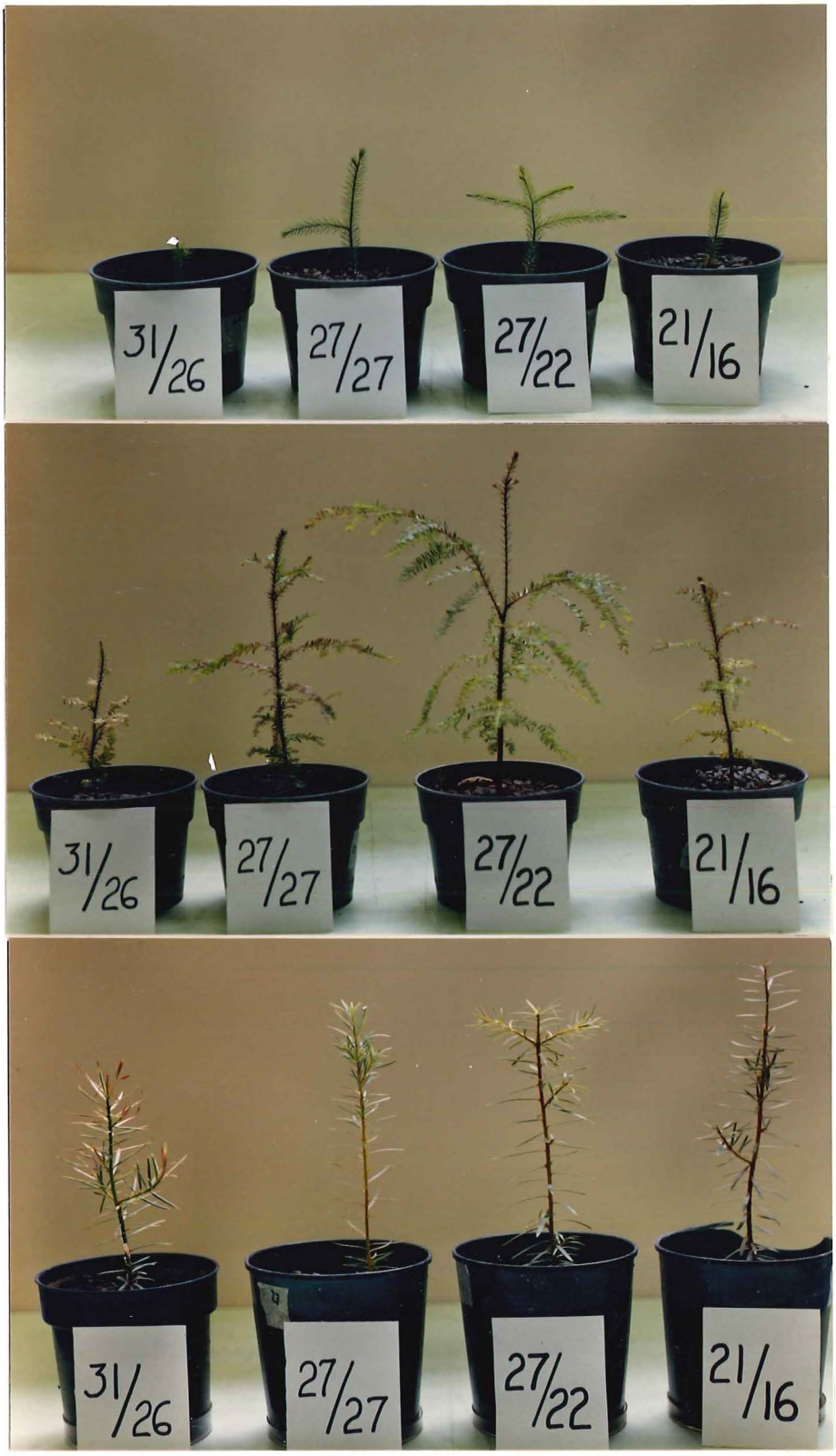

Plate 8: Average seedlings of rimu (top), kahikatea (centre) and totara (bottom) from the second temperature experiment (continued overleaf)

Plate 8: (Continued) Average seedlings of kauri (top) and mountain beech (bottom) from the second temperature experiment

As in the first nutrition experiment, rimu put a significantly higher proportion of assimilate into root tissue than did kahikatea or totara in both temperature experiments. This may be a strategy of this species to make it more competitive in dry or infertile conditions. This strategy would presumably aid survival but apparently prejudices growth rate.

Rimu, kahikatea, totara and kauri had a fairly similar response to temperature, while beech reacted somewhat differently. Kauri did not behave as expected, and its response to increased temperature was less than those species from more 'temperate' environments.

In beech, there was a marked reduction in growth at temperatures above the $27^{o}/22^{o}C$ regime, while for the other species, the decrease was more gradual. All species, however, performed poorly in the $31^{o}/26^{o}C$ regime and very poorly at warmer temperatures. Hellmers (1966) found that a $35^{o}C$ day killed most redwood seedlings, and Hellmers et al. (1970) experienced the same type of losses with Engelmann spruce at a $35^{o}C$ temperature. In that experiment, water was in ample supply, as it was in the experiment under discussion, so the losses had to be due either directly to heat or to the inability of the seedlings to transport water rapidly enough to the tops. Treshow (1970) explains that maximum temperatures which a plant can endure are dictated by the ability of the component proteins and lipids to maintain their structure. As temperatures increase, bonding within these molecules becomes loose, and around $30^{o}C$, sulfhydryl and hydrogen bonds tend to come apart, disrupting enzymes and denaturing molecules. If high temperatures persist, bonds in the protein molecules become completely disrupted and the enzymes become non-functional (Downs and Hellmers, 1975).

Plants may have adaptations such as pubescence, light coloration, reduced leaf size, or manoeuvrable leaf angle to avoid heat accumulation. As can be seen in Table 18, all

species produced relatively thinner leaves at higher temperatures, and this may be a mechanism to dissipate heat.

Another mechanism to eliminate heat is to increase transpiration to a certain point. In the experiment under discussion, declining growth rates of the trees at the 31°C regime may have been due to energy losses from increased transpiration and respiration outstripping inputs from photosynthesis.

Although it was not significant, mortality in all species declined with light intensity in the 35°C regime, and kauri grew better at lower light levels in warmer temperatures. This may be because seedlings that were protected from direct light did not build up as much heat inside their leaves, and would have had a better chance of dissipating any excess heat and surviving.

The varying degrees of response of the five species to the higher temperatures may reflect their varying abilities to deal with or prevent heat accumulation. The larger, darker leaves of beech may have intercepted more radiation than the other species, and the resulting increase in respiration and transpiration may have outweighed photosynthetic inputs, leading to an early decline in growth.

Response in Net Photosynthesis

Table 19 shows that most species increased net photosynthesis between 21°C and 27°C, while beech experienced a decrease. The work of I. McCracken (pers. comm.) corroborates this finding of an optimum temperature of 21°C for net photosynthesis in mountain beech. Considering the range of this species, it would be expected to have a lower temperature optimum than the conifers. In the 31°C temperature regime, all species had decreased net photosynthetic rates.

Compared to many other temperate coniferous species, a 27°C optimum temperature for net photosynthesis seems high.

Doehlert and Walker (1981) found photosynthesis in Douglas-fir to have an optimum temperature range of 10° to 15°C, well below the optimum temperatures for overall growth. Gowin et al. (1980) found the maximum photosynthetic rates of Scots pine and European larch to be at 17°C, in this case the same temperature as maximized relative growth rates. For Abies sachalinensis and Picea glehnii, temperatures of 17° to 25°C and 17°C respectively optimized photosynthesis (Sakagami and Fujimura, 1981). Loblolly pine, a species from areas with hot summers, was found to have higher optimum temperatures for photosynthesis than rimu, kahikatea and totara, with maximum photosynthetic rates occurring at 30°C (Kramer, 1957).

As a component of growth, optimum temperatures for photosynthesis must be subjected to selection pressures. Bannister (1976) records that optimum temperatures are closely correlated with the climate of a species' origin; thus tropical trees have an optimum temperature for photosynthesis of 25° to 30°C, temperate deciduous trees 15° to 25°C, and temperate evergreen trees 10° to 25°C.

Temperature optima may even vary within a species between environments. Some species of trees that grow at subalpine timberlines and at lower altitudes have a 2° to 3°C difference in the optimum temperature for photosynthesis over 1000 m of elevation (Precht et al., 1973).

Other researchers have found that optimum temperatures for photosynthesis and growth within a species change over the growing season. Golomazora (1981) found that the temperature optimum for photosynthesis in Scots pine, Norway spruce, Pinus sibirica and Larix sibirica increased by 5°C over the summer. He concluded that these optima were determined more by ambient temperature than by species.

Sakagami and Fujimura (1981) also found an 8°C increase in the optimum temperature for photosynthesis from February to August in Abies sachalinensis and Picea glehnii.

These results however, are for species that experience much more severe seasonal changes in climate than New Zealand species. As the two temperature trials spanned a six month period including most of the effective New Zealand growing season, it is thought that these species maintain a fairly constant temperature optimum for photosynthesis and seedling growth throughout the growing year.

INTRODUCTION TO THE THIRD TEMPERATURE EXPERIMENT

The first two temperature experiments demonstrated that all five New Zealand forest tree species tested had an optimum growing temperature of 27°C. Even the mountain beech seedlings which were from a higher elevation and therefore a cooler climate exhibited this optimum. The podocarp species, however, had all originated from sites of low elevation and relatively equable climate. The podocarps tested were also 'climax' species, and therefore may not have been subject to strong selection pressures for growth rate. For this reason, a final test of a podocarp species with a 'pioneering' habit which might have been under strong selection pressures to modify its optimum growing temperature to suit its native climate was required. The third temperature experiment aimed to investigate the optimum temperature for growth and net photosynthesis of a subalpine podocarp species, snow totara.

MATERIALS AND METHODS - THIRD TEMPERATURE EXPERIMENT

The third temperature experiment was divided into two sections. First, a preliminary trial of a few individuals under a wide temperature range was carried out so that a narrower range of likely optimal temperatures could be chosen for the full experiment.

Preliminary Trial

For the preliminary trial, 12 snow totara plants, grown from cuttings taken in the summer of 1985 from the Mt. Cheeseman ski field road (Appendix B), were used. Since 1985, these rooted cuttings had been grown outdoors in Christchurch and they were potted into 1.75 l pots in Odering's three month potting mix prior to the trial.

On June 8, 1988, the cuttings were measured for total branch length, height, diameter at soil level, number of branches and saturated pot weight. They were divided into four size classes on the basis of total branch length. The cuttings were then air freighted on June 14, 1988 to the DSIR, Palmerston North where the three cuttings in each size class were randomly allocated to one of three C.E. rooms (Robotham et al., 1978).

The three C.E. rooms were being used for a DSIR flowering experiment, but had surplus space. They were set at constant temperatures of 30°, 22° and $14^{\circ}C$. The lights in each C.E. room were on for 12 hours each day and had intensities of 725, 703 and 705 $\mu molm^{-2}s^{-1}$ respectively. The relative humidity regimes of the C.E. rooms were 89.4, 83.0 and 71.8 percent respectively. All seedlings were watered for 1.5 minutes, three times per day with a modified half-strength Hoagland's solution (Appendix P). A pot of five newly germinated kahikatea seedlings was included as a 'control' in each C.E. room.

After 13 weeks in the C.E. rooms, the cuttings were air freighted back to Christchurch. The cuttings were measured for the same variables as at the start of the trial, however only total branch length, diameter at soil level, and total number of branches were used to compare the growth of the cuttings in the three temperature regimes. The total increase and the increase as a percentage of the starting value of these variables was compared by an analysis of

variance. The model was as follows (see Appendix Q for sums of squares).

SOURCE OF VARIATION	df
Temperature Treatment	2
Error	9
Total	11

The means of the measured variables in each temperature treatment were compared by Scheffe's test, with a confidence level of 0.95.

Third Experiment

The results from the preliminary trial were very helpful in determining the temperature regimes for the full experiment. For this experiment, 600 cuttings were struck in April, 1988 from 10 snow totara plants from each of four locations, and from several totara trees from two locations.

The lower leaves were removed from the cuttings, a wound was made on one side of the bared stem, and the wound dipped in Seradix 3 rooting hormone. The cuttings were then planted in PB 1 1/2 plastic planter bags, five to a bag, in a 2:1 mixture of peat and sand. The planted cuttings were placed in a plastic 'tent' on a heated glasshouse bench, and kept moist for five months.

At the end of September, 1988, the cuttings were carefully removed from the soil mix, and those that had formed roots were planted into 0.25 l pots in Odering's three month potting mix. At this stage there were 26 snow totara cuttings from the Whakapapa location, 12 from Hanmer, 40 from Temple Basin High, 28 from Temple Basin Low, 24 totara cuttings from Waipoua and 26 from Kaikoura (see Appendix B for details of cutting origins).

The newly potted cuttings were kept in the plastic 'tent' for one week, and were then placed on an unheated glasshouse bench for several weeks. At this time, the cuttings were measured for height and number of branches, and

were then divided into 5 cm height classes within each provenance. The seedlings in each size class in each provenance were then randomly divided into two groups. As in the previous experiments, this systematic distribution was done in order to minimize the effects of initial cutting size on the final analysis.

On Nov. 2, 1988, the rooted cuttings were transported to the Lincoln College Plant Science Department. The two groups of plants were randomly assigned to two C.E. cabinets there, and a pot of five newly germinated kahikatea seedlings was included as a 'control' in each C.E. cabinet. The cuttings were arranged randomly within each C.E. cabinet, and their positions were re-randomized every two weeks.

The day/night temperature regimes of the two C.E. cabinets were $27^{o}/22^{o}C$ and $21^{o}/16^{o}C$. The C.E. cabinets were set for a 16 hour photoperiod, synchronized with the temperature cycle, with three hours of only incandescent light before and after the ten hours of full light. Daytime light intensity averaged 305 $\mu molm^{-2}s^{-1}$ in the cabinets. The plants were watered to excess with a hand-held sprinkler every two days, and the relative humidity in the cabinets fluctuated from 45 to 90 percent with the watering regime.

One day before the end of the experiment, five plants from each provenance in each C.E. cabinet were measured for net photosynthetic rate. Measurements were taken with the LI-6200 Portable Photosynthesis System as per the methods of the second temperature experiment. The LI-COR System had been updated between the two temperature experiments, therefore only a 5 ppm drawdown in CO_2 concentration was required to measure net photosynthesis in the third temperature experiment. Net photosynthetic rates were calculated on a total leaf surface area basis, and leaf area was measured with a Delta-T Leaf Area Meter.

After 60 days, the experiment was terminated, and all cuttings were removed to cold storage. The cuttings were

then measured and dissected by provenance.

As previously mentioned, the cuttings had been measured for height and branch number at the start of the experiment, and these measures were taken once during the experiment, and again at the end. The cuttings were dissected into root, stem and leaf portions, and these portions were oven-dried at 80°C for 48 hours and weighed.

The measures of height, number of branches, total, root, stem and leaf weights, and the ratios of these portion weights to total weight were compared, by species, using analysis of variance. As there were unequal numbers of observations, the General Linear Model Procedure (SAS, 1985) was used. The model for snow totara seedlings was as follows (see Appendix R for sums of squares):

SOURCE OF VARIATION	df
Temperature Treatment	1
Provenance	3
Size Class	8
Error	101
Total	113

The interaction terms were not significant and so were pooled with the 'Error' to increase precision.

The means of the provenance terms were compared using Scheffe's test with a confidence level of 0.95.

The photosynthetic rates of the cuttings of both species in the two temperature regimes were also compared using analysis of variance, and the means compared by Scheffe's test. The model was similar to the one above, however a 'Species' term was included and the 'Size Class' term omitted (see Appendix R for sums of squares).

RESULTS OF THE THIRD TEMPERATURE EXPERIMENT

Preliminary Trial

Results of the preliminary trial showed there to be a significant ($p=0.001$) effect of temperature on the growth of

snow totara cuttings. For all measured variables, growth was greatest in the 22°C regime and least in the 14°C regime (Plate 9). The growth of the kahikatea 'control' seedlings was also much greater at 22°C, being three times that at either of the other temperatures. For all measured variables, the growth of the snow totara cuttings at 22°C was not significantly different from that at 30°C, but growth at these two temperatures was significantly greater than at 14°C (p=0.05) (Table 20).

Plate 9: Individual snow totara cuttings before (top) and after (bottom) 13 weeks growth in three constant temperature regimes

The increases in total number of branches and branch length were due primarily to an increase in the number and length of third order branches. The cuttings grew very little in height or in the number or length of primary branches. The saturated pot weights could not be used to measure growth as the pots had been tipped in transport and some soil was lost.

Table 20: Several measures of growth of snow totara cuttings in three temperature regimes

Temperature (^{o}C)	14	22	30
Increase in Total Branch Length (mm)	4.5	5381.0	4657.0
% Increase in Total Branch Length	0.64	250.82	225.50
Increase in Total Number of Branches	0.75	193.5	164.5
% Increase in Total Number of Branches	4.89	428.11	342.20
Increase in Diameter (mm)	0.77	2.30	1.84
% Increase in Diameter	13.92	43.32	36.20

Third Experiment

(i) Growth. For most measures of growth, both species tended to have superior growth in the 27^{o}C regime (Table 21). The difference in growth between temperatures was significant only for diameter ($p=0.02$) in totara. Cuttings of snow totara produced significantly greater ($p=0.0001$) root weights in the 27^{o}C regime, and leaf weights also tended to be higher ($p=0.09$) in the warmer regime. This resulted in a significantly greater total weight for snow totara cuttings in the 27^{o}C C.E. cabinet than in the 21^{o}C cabinet ($p=0.02$) (Plate 10).

The kahikatea 'control' seedlings grew significantly ($p=0.05$) larger in the 27°C regime, averaging 114 cm in height and 13 branches; compared to the average 56 cm height and 9 branches of seedlings in the 21°C regime.

Table 21: Measures of growth of snow totara and totara cuttings in the third temperature experiment

	SNOW TOTARA			TOTARA	
Temperature (°C)	27/22	21/16		27/22	21/16
TOTAL WEIGHT (g)					
Whakapapa	0.2464	0.2184	Waipoua	0.2548	0.2093*
Hanmer	0.1559	0.1518	Kaikoura	0.5721	0.5289
Temple Basin Hi	0.2170	0.1880*	Mean	0.4198	0.3755
Temple Basin Lo	0.2747	0.2300*			
Mean	0.2345	0.2036*			
LEAF WEIGHT (g)					
Whakapapa	0.1437	0.1283	Waipoua	0.1440	0.1229
Hanmer	0.0905	0.0983	Kaikoura	0.2970	0.2897
Temple Basin Hi	0.1124	0.0997	Mean	0.2236	0.2096
Temple Basin Lo	0.1459	0.1273			
Mean	0.1272	0.1143			
ROOT WEIGHT (g)					
Whakapapa	0.0593	0.0434	Waipoua	0.0658	0.0514*
Hanmer	0.0370	0.0291**	Kaikoura	0.1476	0.1368
Temple Basin Hi	0.0600	0.0442**	Mean	0.1083	0.0958
Temple Basin Lo	0.0699	0.0516**			
Mean	0.0604	0.0447**			
HEIGHT (mm)					
Whakapapa	60.1	58.1	Waipoua	69.9	62.0
Hanmer	42.0	40.7	Kaikoura	102.2	96.1
Temple Basin High	54.9	53.8	Mean	86.7	80.0
Temple Basin Low	61.2	63.5			
Mean	56.6	56.3			
DIAMETER (mm)					
Whakapapa	1.93	1.90	Waipoua	1.74	1.61*
Hanmer	1.87	1.93	Kaikoura	2.06	1.96
Temple Basin High	1.69	1.78	Mean	1.91	1.80*
Temple Basin Low	1.91	1.76			
Mean	1.83	1.82			

* Difference between temperatures is significant ($p=0.05$)
** Difference between temperatures is significant ($p=0.01$)

Plate 10: Average final size of snow totara cuttings from the Temple Basin Low (left) and Whakapapa (right) provenances grown in two temperature regimes

The totara cuttings were significantly taller ($p=0.0001$) and had significantly greater dry weights ($p=0.0001$) than the snow totara cuttings. For these measures the cuttings of the Kaikoura provenance were significantly ($p=0.001$) larger than those of the Waipoua provenance.

The four provenances of snow totara reacted in a similar manner to the two temperature regimes. There were significant differences in size among the provenances, but these related to initial differences in cutting size rather than to differences in growth rate. Cuttings from the Temple Basin Low and Whakapapa provenances were significantly ($p=0.05$) heavier and taller than those from the Hanmer provenance. Cuttings from the Whakapapa and Temple Basin High locations did not show any significant differences in growth.

(ii) Allocation of Resources. In the first two temperature experiments, seedlings of rimu, kahikatea and totara tended to increase the allocation of dry matter to leaves and stems at warmer temperatures. In the third

temperature experiment, both totara and snow totara cuttings had a significantly ($p=0.03$) greater leaf weight ratio in the 21°C regime. Snow totara cuttings also had a significantly ($p=0.01$) greater stem weight ratio under the cooler conditions (Table 22).

In the 27°C temperature regime, snow totara cuttings had a significantly ($p=0.0006$) greater allocation of dry matter to roots than in the 21°C regime. Totara cuttings showed the same trend, but it was not significant (Table 22).

Table 22: Allocation of dry weight to leaves, stems and roots of snow totara and totara cuttings in the third temperature experiment

	SNOW TOTARA			TOTARA	
Temperature (°C)	27/22	21/16		27/22	21/16
LEAF WEIGHT/TOTAL WEIGHT					
Whakapapa	0.5800	0.5951	Waipoua	0.5693	0.5879
Hanmer	0.5904	0.6504	Kaikoura	0.5303	0.5650
Temple Basin Hi	0.5267	0.5367	Mean	0.5490	0.5760*
Temple Basin Lo	0.5365	0.5597			
Mean	0.5481	0.5688**			
STEM WEIGHT/TOTAL WEIGHT					
Whakapapa	0.1796	0.2046	Waipoua	0.1732	0.1643
Hanmer	0.1801	0.1538	Kaikoura	0.2026	0.1825
Temple Basin Hi	0.2010	0.2296**	Mean	0.1884	0.1738
Temple Basin Lo	0.2113	0.2184			
Mean	0.1970	0.2126**			
ROOT WEIGHT/TOTAL WEIGHT					
Whakapapa	0.2404	0.2003*	Waipoua	0.2576	0.2478
Hanmer	0.2295	0.1958	Kaikoura	0.2672	0.2524
Temple Basin Hi	0.2733	0.2337*	Mean	0.2626	0.2502
Temple Basin Lo	0.2521	0.2219*			
Mean	0.2549	0.2186**			

* Difference between temperatures is significant ($p=0.05$)
** Difference between temperatures is significant ($p=0.01$)

The Waipoua provenance of totara had a significantly ($p=0.01$) greater allocation of dry matter to leaves than the Kaikoura provenance, while the Kaikoura cuttings had a

significantly (p=0.001) greater allocation to stems. The Hanmer and Whakapapa snow totara cuttings had a significantly (p=0.05) greater allocation of dry matter to leaves than the cuttings from either Temple Basin location. For the allocation of dry matter to stem in snow totara, the Temple Basin provenances had significantly (p=0.05) greater stem weight ratios than did the Hanmer provenance.

There were no differences in root weight ratio between the provenances of either species.

(iii) Net Photosynthesis. Rates of net photosynthesis in snow totara and totara cuttings were significantly affected by temperature (p=0.0003), species (p=0.0002) and provenance (p=0.0001). In all provenances of both species, net photosynthesis was higher in the 27°C regime than in the 21°C regime (Table 23).

Table 23: Mean rates of net photosynthesis in the third temperature experiment

	SNOW TOTARA			TOTARA	
Temperature (°C)	27/22	21/16		27/22	21/16
NET PHOTOSYNTHESIS ($mgCO_2/m^2s$)					
Whakapapa	0.3296	0.3039**	Waipoua	0.3908	0.3586
Hanmer	0.3677	0.2751**	Kaikoura	0.4061	0.3933*
Temple Basin Hi	0.3859	0.3227**	Mean	0.3984	0.3759*
Temple Basin Lo	0.3854	0.3779			
Mean	0.3671	0.3199**			

* Difference between temperatures is significant (p=0.05)
** Difference between temperatures is significant (p=0.01)

The totara cuttings had a significantly greater rate of net photosynthesis than did the snow totara cuttings (p=0.001). Within species, photosynthetic rates of the two totara provenances were not significantly different. The average photosynthetic rates of the four snow totara provenances were generally not very different; however the Temple Basin Low cuttings did have a significantly (p=0.05)

higher rate of net photosynthesis than the Whakapapa cuttings.

DISCUSSION OF THE EFFECT OF TEMPERATURE ON THE GROWTH OF SNOW TOTARA - THIRD TEMPERATURE EXPERIMENT

Subalpine and alpine habitats in New Zealand have only developed in the last few million years, thus the species which now grow in these habitats have evolved relatively recently (Raven, 1973). The alpine species have evolved from their precursors through the processes of adaptive radiation, interspecific hybridization, recombination and self-pollination (Raven, 1973).

Snow totara probably arose from lowland totara species, but is now well adapted to subalpine environments, having the prostrate growth form, reduced leaf size, greater leaf density and greater ability to reproduce vegetatively common in alpine plants (Tranquillini, 1979). Snow totara has also developed a much greater degree of cold tolerance than the other totara species (Sakai et al., 1981). In spite of these adaptations to a more severe environment, snow totara has not evolved an optimum temperature for growth or photosynthesis more suitable to the climates in which it now grows.

Snow totara seems to have maintained the 27°C optimum growing temperature found in rimu, kahikatea and totara. As in totara, snow totara increased dry matter production at 27°C largely through an increase in photosynthetic rate, rather than an increase in leaf production.

All four provenances of snow totara responded similarly, with greater growth at higher temperatures. The differences in size among the provenances were most likely due to the initial size of the cuttings rather than to differences in growth rate. The snow totara provenances had significantly different ($p=0.0001$) heights at the start of

the experiment, and the provenance ranking remained the same throughout the experiment. The snow totara cuttings also had a very slow rate of growth. Therefore, although cuttings have the advantage of being genetically uniform, for this type of experiment with a short duration, care needs to be taken to make the cuttings as similar in size as possible.

In both snow totara and totara cuttings, the allocation of resources to roots increased at warmer temperatures resulting in a decline in the allocation of resources to leaves and, in snow totara, to stems. This contrasts with the first two experiments where increasing temperatures tended to increase resource allocation to shoots and decrease allocation to roots. The discrepancy may be attributable to different allocation responses in cuttings and seedlings. If, when the third experiment was begun, the cuttings had not developed a root system in proportion to the shoot, increased growth due to warmer temperatures may have occurred predominantly in the roots, as root formation in cuttings is enhanced by higher temperatures (Kramer and Kozlowski, 1979). This would have resulted in the increased allocation of resources to roots at warmer temperatures found in the cuttings; whereas the seedlings, which presumably would have had balanced root and shoot sizes at the start of the first two experiments, would have been free to allocate increased resources to shoots under favourable conditions.

The 27°C optimum for net photosynthesis in snow totara is even more striking than the 27°C optimum for growth. The temperature optimum for net photosynthesis is commonly 10° to 12°C or lower in alpine plants (Bidwell, 1974); and many studies have shown there to be altitude-related genetic changes in optimum temperatures for photosynthesis within species and genera (Tranquillini, 1979). This does not appear to be the case for snow totara, as this species has maintained the same optimum temperature for net photosynthesis found in its relatives from lower elevations.

Alpine plants tend to have much higher light saturation values for photosynthesis than plants from low altitudes, and their efficiency at low CO_2 concentrations is also greater (Bidwell, 1974). Plants from higher altitudes also usually show higher respiration rates which may represent a general adjustment of metabolic rate that enables the plants to function effectively at low temperatures (Bannister, 1976). This could possibly explain why rates of net photosynthesis were lower in the snow totara cuttings than in the totara cuttings, however data from more provenances of totara would be needed to support such a conclusion

From the third temperature experiment, it would appear that snow totara has not adapted its optimum temperatures for growth and photosynthesis to suit the habitats in which it now grows. Instead, these characteristics appear to have remained unchanged from those of snow totara's lowland relatives.

CONCLUDING DISCUSSION OF THE THREE TEMPERATURE EXPERIMENTS

As the growth of species at high or low temperatures can be limited by the velocity of a single enzymatic reaction (Downs and Hellmers, 1975), it seems likely that where selection pressures have been for growth rate, rapid adaptation would occur. The literature indicates that most plant species are closely adapted to their climates of origin, and the influence of temperature on the growth and photosynthesis of such plants depends almost entirely on provenance (Gowin et al., 1980).

Table 24 lists the optimum growing temperatures of some forest tree species from around the world, plus the species studied in the three temperature experiments. The table also lists the actual mid-summer temperatures of the locations from which these species originated, and the

differential between the optimum and actual temperatures.

A two-tailed 't' test shows the differentials for the New Zealand species to be significantly greater (p=0.0001) than the differentials of the other forest tree species. Thus the New Zealand forest tree species studied do not appear to have the close adaptation of growth to climate present in many other trees.

Table 24: A comparison of the differential between optimum growing temperatures and actual mid-summer temperatures for forest tree species around the world

Species	Optimum Temp (°C)	Actual Temp (°C)[1]	Differential
Snow totara	27.0	16.3	10.7
Rimu	27.0	16.8	10.2
Mountain beech	27.0	17.0	10.0
Kahikatea	27.0	17.9	9.1
Totara	27.0	21.5	5.5
Kauri	27.0	22.2	4.8
Radiata pine[2]	23.0	21.0	2.0
Redwood[3]	19.0	17.0	2.0
Douglas-fir[4]	21.0	20.2	0.8
River red gum[5]	30.0	31.0	-1.0
Pitch pine[6]	26.0	27.2	-1.2
Loblolly pine[7]	26.5	28.3	-1.8
Scots pine[8]	17.0	19.0	-2.0
European larch[8]	17.0	19.0	-2.0
Western hemlock[4]	18.0	20.2	-2.2
Engelmann spruce[9]	19.0	24.1	-5.1

[1] Climate data relates specifically to the provenances used in the studies (Pearce and Smith, 1984; New Zealand Meteorological Service, 1981; U.S. Environmental Data Service, 1968).
Actual Temp. = (mean temp. of warmest month + mean maximum temp. of warmest month)/2

[2](Hellmers and Rook, 1973)
[3](Hellmers, 1966)
[4](Brix, 1971)
[5](Sa-ardavut et al., 1984)
[6](Good and Good, 1976)
[7](Kramer, 1957)
[8](Gowin et al., 1980)
[9](Hellmers et al., 1970)

It is suggested that the response of these New Zealand tree species is a reflection of their early evolutionary history. Ancestors of today's podocarps were evident in the fossil record of the late Triassic, over 200 million years B.P. (before present) (Miller, 1977). Podocarps and Araucarians were present throughout the Jurassic and early Cretaceous, and fossil evidence of Nothofagus dates back to the Cretaceous (Fleming, 1975). The first appearance of Dacrydium and totara groups was in the late Cretaceous, about 96 million years B.P. (Mildenhall, 1980). Fleming (1975) records the first possible Dacrycarpus fossils to date from the late Jurassic, 140 million years B.P.; however Mildenhall (1980) states that the first pollen evidence of Dacrycarpus comes from the early Eocene, 54 million years B.P. Reliable fossil evidence of the tree we know today as rimu has not been found prior to the Quaternary (Norton et al., 1988), but in spite of the debate over the date of the first appearance of these species, it is obvious that they or their predecessors have been evolving with New Zealand over the last 200 million years.

Two hundred million years ago in the late Triassic, wet, monsoonal climates extended to 50°N and S latitudes. A global warming and drying trend was evident through the Jurassic and early Cretaceous however, and this warming trend continued until the late Cretaceous, approximately 96 million years B.P., when sea temperatures around 'New Zealand' reached a peak of 18° to 20°C (Frakes, 1979). At this time, modern day tropical to subtropical conditions extended as far south as 70°S latitude. Mean annual temperatures would have been 10° to 15°C warmer than at present, and the altitudinal temperature gradient would have been about half of what it is today (Frakes, 1979).

About 120 million years B.P. in the early Cretaceous, 'New Zealand' lay at the south-east corner of Gondwanaland at 80°S latitude (I. Daniel, pers. comm.). Between 60 and 80 million years B.P., when world climates were subtropical,

Gondwanaland began to break apart, and by the end of the Cretaceous, New Zealand had separated from Australia and had begun to migrate northwards. During the Paleocene, Australia separated from Antarctica, and the west wind drift developed which had a marked effect on climates in the area (Fleming, 1975). By the mid-Paleocene, New Zealand had migrated north to span 55° to 65°S latitude (Fleming, 1975).

Throughout the Paleocene and Eocene, the old New Zealand land mass was substantially eroded, and land area was much reduced (Fleming, 1975). At the same time, world climates cooled, and then warmed again to tropical conditions in the mid-Eocene. This was followed again by a cooling trend in the mid-Oligocene, at which stage, 35 million years B.P., most of New Zealand was covered by sea, and ocean temperatures were only slightly warmer than at present (Fleming, 1975).

In the late Oligocene, a slow re-emergence of land began, coupled with a climatic warming trend. The warming trend reached its peak in the late Miocene, with sea temperatures of 18°C (Frakes, 1979) and general subtropical conditions existing on land (Fleming, 1975). At this time, New Zealand reached the limits of its northerly migration.

At the beginning of the Pliocene, 7 to 11 million years B.P., there was a sharp cooling of the world's climate. Major Antarctic ice sheets began to appear 5 million years ago, and the Kaikoura orogeny produced New Zealand's Southern Alps; however even three million years ago the sea temperatures around New Zealand were warmer than today (Stevens, 1985). Two million years ago, a subalpine zone appeared in New Zealand (Fleming, 1975), and about 1.7 million years ago, the last tropical taxa disappeared (Mildenhall, 1980). Soon after, the glaciers began to advance.

It can be seen that during the evolution of today's podocarps and their predecessors, climatic conditions in New Zealand have generally been subtropical to tropical.

Subtropical taxa dominated the vegetation from 65 to 2 million years B.P., and evolution of these species would have taken place under subtropical to tropical conditions. Subtropical conditions would also have existed during the 'expansion phase' in the Miocene when the podocarps would have been recolonizing land emerging from the ocean. Rates of evolution are higher in expanding populations (Levin and Wilson, 1978), and at these times selection pressures would have been adapting these species for growth at high temperatures.

The maintenance of high growing temperatures in the species studied implies that selection pressures in the past two million years have not been for growth. A slow rate of evolution in the tree species could also contribute to their maintenance of historic affinities. The high forest species have long life spans and delayed reproduction and so would be slow to respond to a change in selection regime (Levin and Wilson, 1978). Even snow totara however, which has obviously adapted in some respects to the colder climates of the past two million years, has not lost its adaptation to subtropical growing temperatures. This would indicate that selective forces over the recent past have not put pressure on these species to modify their growth rates, but instead have been acting on other attributes such as cold resistance.

Another interesting example of a species maintaining a previously adaptive trait which is no longer appropriate is that of Salix safsaf in South Africa. This species is a representative of the northern deciduous broadleaf genera which crossed the tropics to be represented in the South Temperate Zone. It survives freezing to -30°C, whereas the Ericas and Proteas native to South Africa scarcely tolerate freezing to -10°C (Sakai and Wardle, 1978). While it is not known how long this willow has maintained its 'unnecessary' frost tolerance, it demonstrates that traits upon which selection pressures no longer act may be maintained over many generations in plant populations.

While the exact historical sequence of continental drift, marine transgression and mountain building is unique to New Zealand, the overall climatic influences are not. The warming trends of the Cretaceous, Eocene and Miocene were global, and subtropical conditions extended over much of the earth (Frakes, 1979). Why then, are the New Zealand conifers so different from their counterparts in the northern hemisphere?

In the Permian, 280 to 225 million years B.P., all continents were united in a single land mass called Pangaea. Pangaea had a comparatively uniform climate, and soon came to contain fairly uniform flora (Cox et al., 1976). In the early Triassic, Pangaea separated into two continents, Laurasia and Gondwanaland, and separate floral provinces began to appear. The subdivision of the continents continued through the Mesozoic, however representatives of the primitive gymnosperms remained in both the northern and southern hemispheres (Cox et al., 1976).

By the beginning of the Tertiary, 60 million years B.P., Europe was still 20^{0} latitude south of present, and North America was also farther south (Wolfe, 1971). The climate of North America at that time was warm and the vegetation had distinctly warm-temperate or tropical affinities (Graham, 1972). Over the Oligocene as climates cooled, this vegetation was replaced by a temperate, broadleaf, deciduous, hardwood forests with the few gymnosperms present being members of the Taxodiaceae. Warming climates in the Miocene were accompanied by a series of orogenies in western North America, and also by a slow, northwards migration of the northern hemisphere continents. These events resulted in the creation of an upland, mesic, coniferous community of members of the Pinaceae approximately 16 million years ago (Graham, 1972).

The sharp climatic cooling of the Pliocene and Pleistocene was much more drastic in the northern hemisphere because of the increased cooling caused by large, continental

masses close to the pole, and Europe and North America were stripped of all tropical and subtropical taxa by the Pleistocene. The negligible land mass and the correspondingly larger body of ocean at the same latitudes in the southern hemisphere had a moderating effect on the climate (Potvin, 1975). Thus the New Zealand forest tree species which were adapted to subtropical climates were able to survive, while their northern hemisphere counterparts could not and were replaced by the newer pines and larches which had had longer to adapt to the colder climates. Douglas-fir, for example, is thought to have evolved as recently as the middle Pleistocene (Silen, 1978), and thus would almost certainly be adapted to the colder climates of that time. The paucity of species in North America and Europe, as compared to the South Pacific, is evidence both of the severity of the colder climates and of the northern species' recent origin (Cox et al., 1976).

During the Ice Ages in North America, the north-south oriented mountain ranges may have acted as 'migration pathways', and this would have helped preserve the entire forest community as it shifted with each climatic change (Silen, 1962). In Europe however, east-west mountain ranges would have blocked migration, and cold sensitive species have been periodically purged, leaving tolerant but slow growing species to recolonize the continent (Silen, 1962). Although Pleistocene and Holocene climatic conditions would not have been as harsh in New Zealand as in the northern hemisphere, during glacial periods there would have been little opportunity for species to 'migrate' because the land area was relatively limited. Thus cold sensitive species or provenances may have suffered the same fate as those in Europe, and the plants left to recolonize the Islands may have been the more cold resistant but slower growing components of the earlier populations.

The evolution of plant species in Australia bears some resemblance to the sequence of events in both New Zealand and

North America. Sixty million years ago when Australia separated from Gondwanaland, the continent was covered by a cool, temperate rainforest dominated by Podocarpus, Dacrydium, and Araucaria species (Florence, 1983). Nothofagus was a minor element of this forest. Until 38 million years ago, migration between Australia and South America was possible via Antarctica and the South Tasman Rise. This route was limited to cold tolerant plants (Boland et al., 1984) thus selection pressures for cold tolerant species existed earlier in Australia than in New Zealand. By 15 million years ago, Australia had migrated northwards and was in close proximity to Asia, and an exchange of species occurred. Thus Australia's flora has been much less isolated that New Zealand's, and there has been immigration of both cold tolerant and tropical species.

The Eucalyptus species of Australia arose in the late Miocene in response to a drying climate, and declining soil fertilities (Florence, 1983). The major effect of the Pleistocene glaciations in Australia was to increase aridity, and Eucalyptus dominated the vegetation during these periods (Boland et al., 1984). At the end of the glaciations, eucalypts continued to dominate in the drier areas, while new, subtropical rainforest species from the Indo/Malaysian region replaced them in east and northeast Australia. The remaining temperate rainforests of Victoria and Tasmania were then the only areas left to act as refugia for the ancient gymnosperms and southern beeches.

Growth studies indicate that many forest tree species of the world have responded to selection pressures by growing as rapidly as their environment will permit, commensurate with long-term survival (Silen, 1962). In contrast, it appears that the ancient New Zealand podocarps, araucarians and beeches may have survived over the last two million years by responding to selection pressure for traits other than growth rate.

CHAPTER V

AN EXPERIMENT TO DETERMINE THE EFFECT OF TEMPERATURE ON THE GROWTH OF SEVERAL PROVENANCES OF TOTARA

INTRODUCTION

In the discussion of the three temperature experiments, it was suggested that the optimal growth of rimu, kahikatea, totara and snow totara at 27°C is a historical adaptation to subtropical conditions prevalent in New Zealand during these species' early evolution. If this hypothesis is correct, and subsequent selection pressures have not favoured growth rate, it would be expected that all provenances of these species would react in the same positive way, experiencing optimal growth at temperatures around 27°C. If, however, selection pressures over the past two to five million years have been acting upon growth rate, a differentiation in optimal growing temperature between different provenances might be expected, with provenances from cooler areas growing optimally at lower temperatures.

A wealth of literature exists concerning provenance-related experiments with trees from around the world. Generally, it is concluded that intra-specific variability exists, and this may or may not be related to climatic or geographic variables. Even species of tropical environments, where selection pressures are thought to be weak, show some variation (Ashton, 1969).

Many different expressions of variability are described in the literature. Morphological variation in leaf size, shape, number and colour, branch angle and crown shape is documented. Other studies investigate the timing of bud burst and set, and flowering. Frost tolerance is another trait often studied, but variability in growth rate, particularly for commercial species, is one of the most

widely documented criteria for provenance comparison.

As would be expected, variability often increases with the breadth of a species' range. Red beech, a species with a narrow ecological range, exhibits only minor variation (Wilcox and Ledgard, 1983). Teak also is a species with low variability in vigour (Suri, 1984), but there are differences between provenances in stem quality and flowering (Delaunay, 1978). Other tropical trees which grow over wider areas but in relatively uniform climates also show low levels of adaptive variation (Ashton, 1969).

When species do exhibit significant inter-provenance variability, this variability is not necessarily related to climate or geography. Western hemlock is a wide-ranging species with a high degree of variability; but while bud burst and set are highly correlated with elevation and latitude of origin respectively, variation in growth rate is random (Pollard and Portlock, 1986; Kuser and Ching, 1980). Eucalyptus camaldulensis also exhibits variation in growth rate that is unrelated to the location of seed origin (Saardavut et al., 1984), and the same is true of Eucalyptus nitens (McKimm, 1985).

Many North American conifers however, particularly the 'pioneer' species, show provenance differences that are strongly correlated with the location of seed origin. Douglas-fir is one of the most intensively studied species, and genetic differentiation of this tree has been found to be strongly related to environmental gradients (Rehfeldt, 1983; Ching and Bever, 1960). Silen and Mandel (1983) found height growth in Douglas-fir to clearly reflect major topographic features over areas as small as 296,500 hectares. Generally, trees from milder environments displayed a higher growth potential but lower hardiness than provenances from severe environments. White et al. (1981) provide a comprehensive list of references for this species.

Other species whose growth varies clinally relative to elevation and latitude are lodgepole pine (Moore, 1984; Rehfeldt and Wykoff, 1981) and white spruce (Dunsworth and Dancik, 1983). It also appears common for growth to be strongly related to elevation alone, and big tree (Guinon et al., 1982), western larch (Rehfeldt, 1982), Eucalyptus regnans (Griffin et al., 1982), and silver beech (Wilcox and Ledgard, 1983) are examples where such adaptation occurs. Other species such as jack pine, Scots pine and Sitka spruce show a strong correlation of growth with latitude (Mergen et al., 1974); while the growth of black spruce is correlated with longitude (Fowler and Park, 1982).

A set of totara seedlings of various provenances grown in glasshouse and field conditions showed large, within-provenance differences in response to the two environments (G.B. Sweet, pers. comm.). It appeared that the different temperature regimes were causing the variations in the growth of the seedlings, and yet this provenance X temperature interaction seemed to bear no relationship to the climates of seedling origin. If selection pressures of the recent past have been acting upon growth rates, it could significantly affect species' distributions and dominance patterns; therefore an experiment was designed to test the provenance X temperature interaction and to discover whether the optimal growing temperatures of seven provenances of totara bore any relation to their climates of origin.

MATERIALS AND METHODS

Seedling Origins

On Oct. 21, 1986, one-year old seedlings of 21 totara provenances were air-freighted to the University of Canterbury from the Forest Research Institute, Rotorua, by courtesy of D.O. Bergin. These seedlings were from seed collected during a nation-wide project in 1985, and had been

germinated in 1985 and grown outdoors for one year (Bergin and Ecroyd, 1987).

The seedlings were potted into PB 1 1/2 plastic planter bags in Odering's Nine Month Potting Mix, and were placed on capillary benches in a glasshouse. In the glasshouse, temperatures averaged 23°C and ranged between 15° and 35°C; while relative humidity averaged 60 percent and ranged between 25 and 90 percent.

On April 3, 1987, 28 seedlings of seven provenances from east of the main ranges were chosen. Details of origin are presented in Table 25.

Table 25: Totara provenance numbers, locations and elevations and latitudes of origin (see Appendix B for further details)

Number	Location	Elevation (m)	Latitude
T041	Kaikohe	240	35° 21′
T105	Waiotahi	70	38° 07′
T059	Gisborne	50	38° 27′
T120	Masterton	210	40° 49′
T129	Ngaumu	370	41° 01′
T111	Kaikoura	240	42° 17′
T097	Peel	300	43° 54′

The 28 seedlings of each provenance were divided into seven groups of four seedlings by estimated total weight. These four seedling groups were further divided into two similar pairs, and the two pairs were randomly designated 'warm' or 'cool'. Of each pair, one seedling was potted into a 1.75 l pot in Odering's Nine Month Potting Mix. The other plant in each pair was measured for height, number of branches and stem diameter at soil level, and then harvested. The harvested seedlings were divided into root, stem and leaf portions; and these tissues were oven-dried at 80°C for 48 hours and weighed.

Controlled Environment Conditions

The newly potted seedlings were placed in two C.E. cabinets (Temperzone, Auckland) in the Controlled Climate Laboratory of the Forest Research Centre, Christchurch (Plate 11).

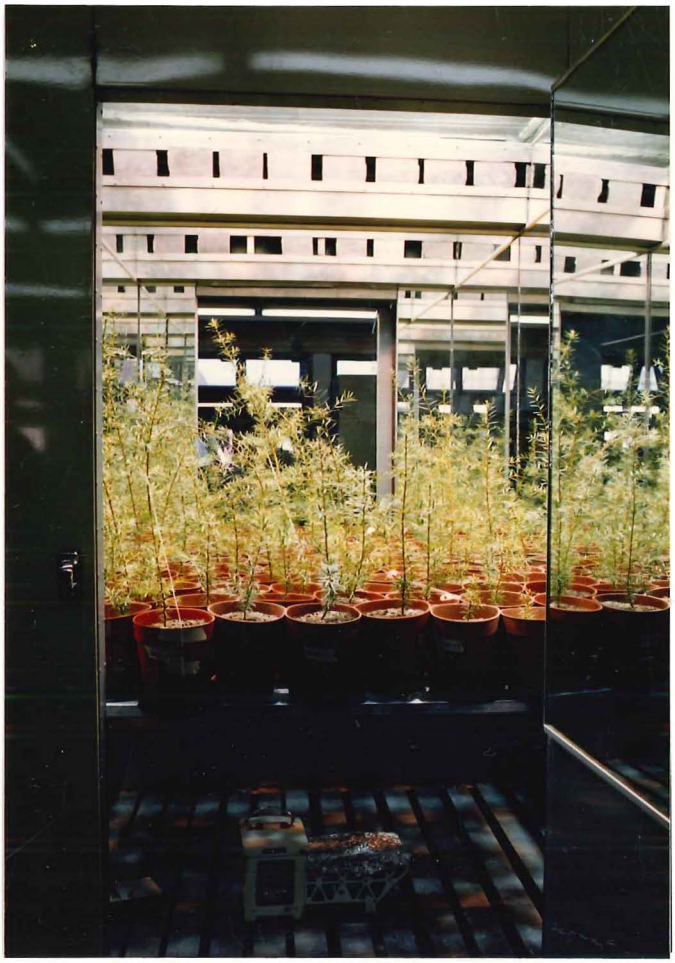

Plate 11: Seedlings of the seven totara provenances in the $21^{o}/16^{o}C$ C.E. cabinet

The seedlings designated 'warm' were placed in a $27^{o}/22^{o}C$ temperature regime, while the 'cool' plants were placed in a $21^{o}/16^{o}C$ regime. The photoperiod was 16 hours with 3 hours of only incandescent light at either end of a 10 hour period of full light. The relative humidities in the

cabinets were set at 68 and 55 percent respectively to maintain a maximum vapour deficit of 12 mb. Full light intensities were 500 $\mu molm^{-1}s^{-2}$ in the 'warm' cabinet and 650 $\mu molm^{-1}s^{-2}$ in the 'cool' cabinet.

Measurements of Photosynthesis and Growth

The experiment ran for 60 days. At the end of this time, four pairs of seedlings ('warm' and 'cool') from four provenances were measured for photosynthetic rate with the LI-6000 Portable Photosynthesis System (see Materials and Methods - Second Temperature Experiment).

The temperatures in both cabinets were then reduced to a $15^{o}/10^{o}C$ regime to minimize further growth, and the plants were harvested as rapidly as possible. The seedlings were harvested size class by size class across all provenances and temperatures. They were measured for height, number of branches and diameter at soil level. The seedlings were then divided into root, stem and leaf portions; and these tissues were oven-dried at $80^{o}C$ for 48 hours and weighed.

Analysis

(i) Analyses of Variance. The first step in analyzing the results was to carry out an analysis of variance for all final values of the measured growth variables, total weight, root weight, stem weight, leaf weight, height, number of branches and stem diameter. The General Linear Models Procedure (SAS, 1985) had to be used as there were unequal numbers of observations. The model was for a split-plot design as follows (assuming no missing observations):

	SOURCE OF VARIATION	df
Whole plot	Temperature Treatment	1
	Size Class	6
	Error A	6
Split-plot	Provenance	6
	Provenance X Treatment	6
	Provenance X Size Class	36
	Error B	36
	Total	97

This analysis did not show up any significant provenance X temperature interactions, apparently because of the large variation in mean provenance size. Therefore another analysis was carried out using the growth in each measured variable where:

Growth = (Value at $time_{60}$ - Value at $time_0$)

The same ANOVA model as before was used, but again results were not clear cut. For this reason another analysis of variance was done. This time the difference in the values of growth for each measured variable between the 'warm' and the 'cool' cabinets was analyzed. This value was calculated as follows:

Difference in Growth = [(Value at $time_{60}$ - Value at $time_0$ at 27°/22°C) - (Value at $time_{60}$ - Value at $time_0$ at 21°/16°C)]

The model for this analysis was as follows (assuming no missing observations) (see Appendix S for sums of squares):

SOURCE OF VARIATION	df
Provenance	6
Size Class	6
Error	36
Total	48

The results from this analysis were more positive with some variables showing significant differences in growth between 'warm' and 'cool' cabinets.

The photosynthetic measurements were compared with an analysis of variance. The model for this analysis was as follows:

SOURCE OF VARIATION	df
Temperature Treatment	1
Provenance	3
Size Class	3
Treatment X Provenance	3
Treatment X Size Class	3
Provenance X Size Class	9
Error	9
Total	31

(ii) Regressions. Finally, regressions were calculated to provide another way of comparing provenance response.

For each measured growth variable, the individual seedling values at $time_0$ were taken and the 'warm' cabinet seedling value was divided by the corresponding size class 'cool' cabinet seedling value. For instance:

$$\text{Value for Analysis}_0 = [(\text{Value at time}_0)_{27^o/22^oC} \;/\; (\text{Value at time}_0)_{21^o/16^oC}]$$

The same thing was done for the individual seedling values at $time_{60}$.

A regression was calculated for each provenance of the seedling pair ratios (Value for $Analysis_0$ and Value for $Analysis_{60}$) against time. Each provenance, therefore, had a best-fit line fitted between the seven seedling pair ratios at $time_0$ and the seven seedling pair ratios at $time_{60}$. The provenance regression lines were tested for the hypothesis that their slopes were equal to zero. If the slope was equal to zero, this meant that the plants grew equally well in 'warm' or 'cool' conditions. If the slope was positive and significant, it meant the seedlings of that provenance grew better in the 'warm' environment. Conversely, if the slope was negative, the plants grew better in cooler conditions. This offered a very clear way of comparing growth of provenances of varying size at different temperatures (see Appendix T for slopes, intercepts and their respective probabilities for the significant regressions). The individual slopes of each provenance's regression line were also compared with each other using covariance analysis.

(iii) Climatic and Geographic Correlations. As the regression analyses displayed different optimum growing temperatures for different provenances, an attempt was made to correlate variation in growth with the elevation, latitude, days of ground and air frost, mean October to March rainfall and temperature of seed origin. These climatic and

geographic variables were correlated with the provenances': (1) absolute growth in total weight and growth as a percentage of starting weight in the $27^{o}/22^{o}C$ regime, (2) growth in total weight and growth as a percentage of starting weight in the optimal regime, and (3) the difference in height growth between the 'cool' and 'warm' regimes. The correlations were done, first including all provenances, and then with all provenances except T097. The seedlings of provenance T097 were excluded as they were heavily infested with a scale insect.

(iv) Data Validation. To ascertain whether initial differences in seedling size influenced the regression slope, an analysis of variance was carried out for each provenance for the values of individual seedling relative growth rate in the two cabinets. The model was as follows:

SOURCE OF VARIATION	df
Size Class	6
Temperature Treatment	1
Error	6
Total	13

In no case was growth within the provenances significantly affected by size class; thus the regression method of comparing provenance growth was valid.

A non-parametric analysis was also carried out on the initial and final values of dry weight to ascertain that the distributions of the data from the various provenances were equivalent.

(v) Verification of Results. The results of this experiment were compared with Slui's (1988) results for the growth of 19 of the 21 F.R.I. totara provenances grown at Rangiora and in the glasshouse. Some recalculation of Slui's (1988) data was performed as described in the discussion.

RESULTS

Analyses of Variance

The analysis of variance of the variables measured at the end of the experiment showed the effects of size class and provenance on growth to be significant ($p=0.001$). This demonstrated the effectiveness of the division of seedlings into size classes, and emphasized the inter-provenance differences (Plate 12).

The effect of temperature on growth was not always significant. Total weight distribution showed little relation to temperature. Final height, however, was significantly ($p=0.03$) affected by temperature with the tallest plants in the cooler cabinet. Number of branches and leaf weight were also affected by temperature ($p=0.08$), and these parameters were at a maximum in the $27^{o}/22^{o}C$ regime.

The provenance X temperature interaction was not significant for any of the measured variables, but this was not surprising as initial seedling size was so variable, and had such a great effect on the final measurements. For this reason, more sensitive analyses of inter-provenance differences in growth were carried out.

The analysis of variance of actual growth in the measured variables (value at $time_{60}$ - value at $time_{0}$) showed much the same trends. Provenance affected growth of all variables significantly ($p=0.001$). Growth in height was significantly ($p=0.01$) affected by temperature with plants being, on average, 44 cm taller in the $21^{o}/16^{o}C$ temperature regime. The increase in primary branch number was significantly ($p=0.02$) greater at warmer temperatures with those seedlings averaging 4.3 more branches than their 'cool' regime counterparts. Leaf weight was accordingly 0.5 g greater, on average, in the warmer cabinet. Growth in stem diameter was the only variable for which the provenance X temperature interaction was significant ($p=0.03$).

Plate 12: Average seedlings of the seven totara provenances at the start of the experiment (top)
Maximum within-provenance variation in seedling size for largest and smallest provenances (centre and bottom respectively)

A further analysis of variance by provenance using the difference in the growth of variables between the 'warm' and 'cool' cabinets produced some interesting trends. Table 26 displays the data for the growth and net photosynthetic measurements, as the difference in growth between the 'warm' cabinet and the 'cool' as a percentage of growth in the 'cool'. Only the differences in stem diameter growth were significant ($p=0.03$).

General trends showed that all provenances grew taller in the cooler regime. Root growth was also greatest in the cooler temperatures, except for T120. T120, T129 and T097 generally seemed to perform better at the warmer temperatures, while T105, T059 and T111 appeared to grow better in the cooler regime. T041 appeared quite impartial to temperature. It can also be seen that provenances T120 and T097 had a higher photosynthetic rate in the 'cool' cabinet, while the opposite was true of provenances T105 and T041.

Regressions

To further assess differences in growth, regressions were calculated by provenance for the ratio of 'warm' to 'cool' values of measured variables at the beginning and end of the experiment. The slopes of these regressions were compared.

The slopes of the provenance's regression lines were significantly different for total weight ($p=0.10$), height ($p=0.05$), root weight ($p=0.05$) and stem diameter ($p=0.01$). In terms of weight, (Figure 11), T120 increased significantly faster in the 27/22°C regime, while T059 increased faster in the 21°/16°C regime. The other provenances showed no significant differences in growth between temperatures.

Table 26: Differences in the growth of measured variables and net photosynthesis between the 'warm' and 'cool' C.E. cabinets as a percentage of that in the 'cool' for seven totara provenances

	Provenance	% Difference
TOTAL WEIGHT (g)	120	39.9
	097	13.4
	129	7.3
	111	- 1.0
	041	- 4.1
	059	- 8.1
	105	- 8.3
ROOT WEIGHT (g)	120	33.6
	097	- 10.1
	129	- 11.5
	041	- 12.2
	111	- 26.7
	105	- 31.8
	059	- 32.0
LEAF WEIGHT (g)	120	45.0
	097	26.6
	129	18.2
	111	9.7
	041	1.0
	059	- 2.4
	105	- 11.0
HEIGHT (mm)	097	- 3.7
	059	- 6.9
	105	- 7.7
	120	- 14.3
	041	- 25.6
	111	- 26.7
	129	- 27.0
DIAMETER (mm)	120	56.8
	105	8.6
	129	3.5
	097	- 0.7
	041	- 3.4
	111	- 9.6
	059	- 20.1
NET PHOTOSYNTHESIS	041	6.8
	105	3.7
	097	- 3.6
	120	- 10.9

Growth in stem diameter offered the highest level of significance (p=0.01) for differences in growth between temperature regimes. The trends for these regressions were identical to those in Figure 11. T059 increased stem weight more quickly in the cooler regime, but the other provenances grew equally well at either temperature regime.

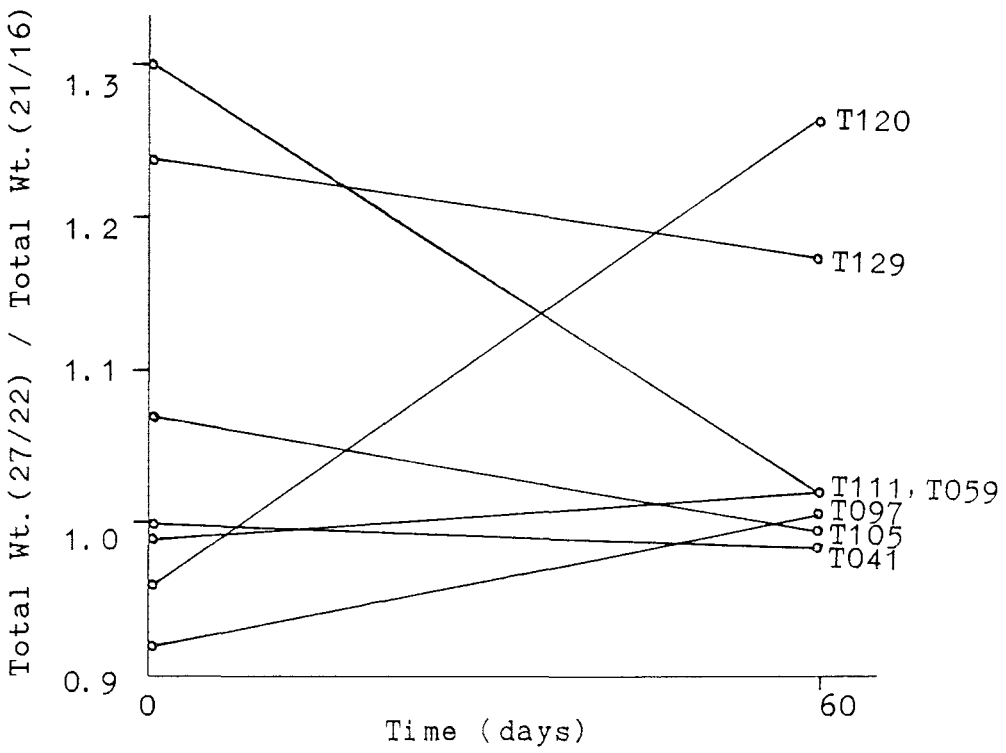

Figure 11: Plot of the ratio of total weight at 27°/22°C and total weight at 21°/16°C versus time

Although seedlings of all provenances grew taller in the cooler cabinet, T105, T111, T129 and T041 grew significantly (p=0.10) taller in the 21°/16°C regime. T120, T097 and T059 did not show a significant difference in height growth from the 27°/22°C regime (Figure 12).

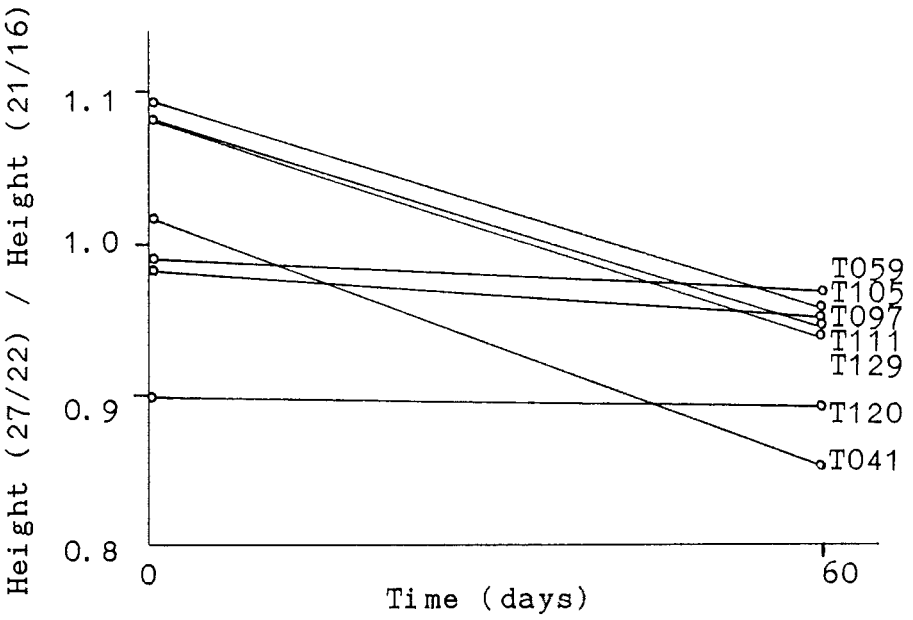

Figure 12: Plot of the ratio of height at 27°/22°C and height at 21°/16°C versus time

Growth in root weight (Figure 13) was greatest in the cooler cabinet for T105, T041 and T059; while T120 was the only provenance to have superior root growth at warmer temperatures. The trends in the change of the ratios of leaf weight to total weight between 'warm' and 'cool' regimes (Figure 14) show that all provenances except T105 increased their allocation of resources to leaves in the warmer temperatures. T041 and T097 were the only provenances where the increase was significant.

Net Photosynthesis

There were no significant differences in photosynthetic rates between temperature regime or provenance accompanying the increases in leaf weight in the four provenances tested.

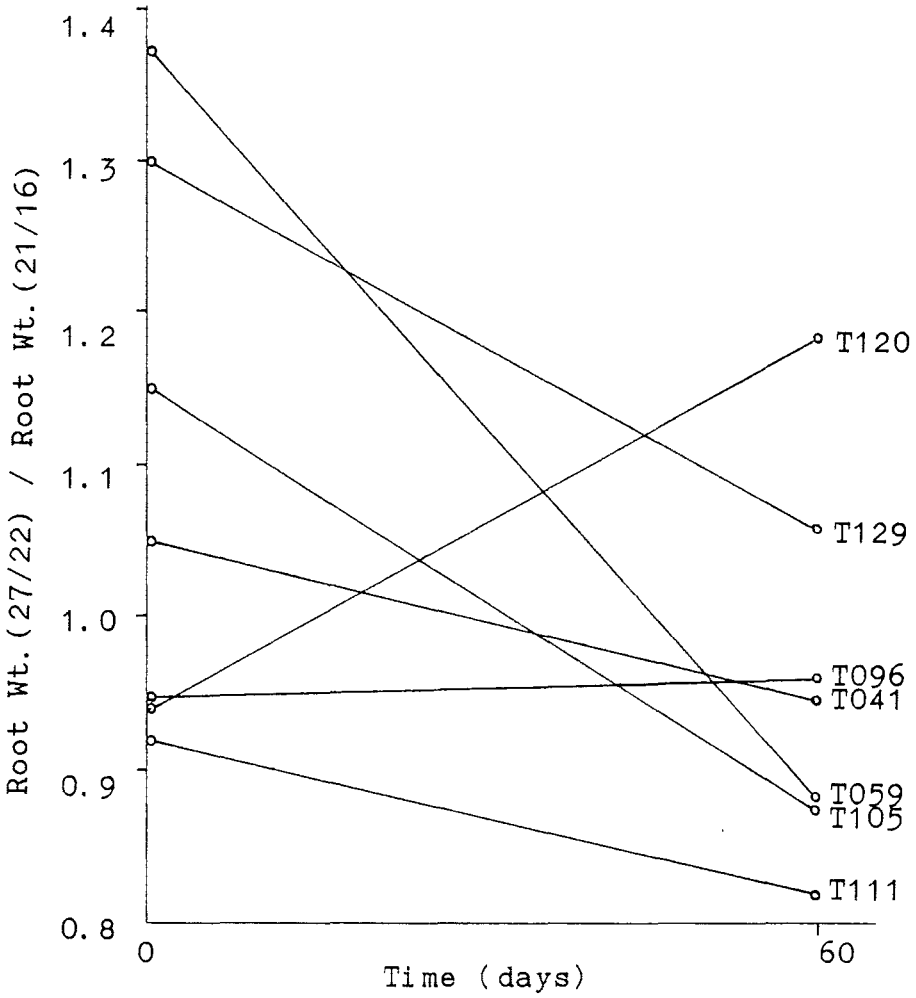

Figure 13: Plot of the ratio of root weight at 27°/22°C and root weight at 21°/16°C versus time

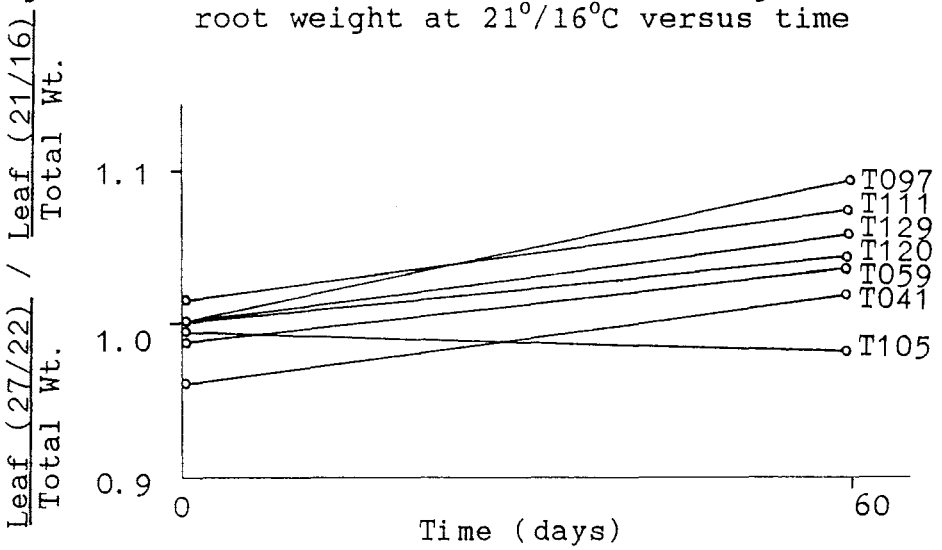

Figure 14: Plot of the ratio of the allocation of dry matter to leaves at 27°/22°C and 21°/16°C versus time

Climatic and Geographic Correlations

An attempt was made to relate the differences in growth rates to the climates of the provenance's origins. Growth in the 'warm' cabinet, and maximum growth for each provenance were separately compared with a series of geographic and climatic variables; however when all seven provenances were included, no significant correlations were found. If the data from provenance T097 were removed however, several correlations became significant. The justification for removing this provenance is that these plants were infested with a scale insect which was not discovered until the end of the trial, and this may have reduced their growth.

Without T097, absolute growth of the six provenances was significantly and positively correlated with latitude and days of ground frost, and negatively correlated with temperature ($p=0.01$). Percentage growth, however, had no significant correlations. The difference in height growth between the 'cool' and 'warm' cabinets was positively correlated with elevation ($p=0.006$).

This trial demonstrated that growth of some of the seven totara provenances was significantly affected by temperature, with the overall optimum temperature varying between provenances. T120 performed best in the $27^{o}/22^{o}C$ regime, and T097, and perhaps T111, also seem to follow this trend. T059 performed best in the $21^{o}/16^{o}C$ regime, and T129, T105 and T041 also appeared to prefer cooler temperatures.

Slui (1988) collected and collated data on the growth of seedlings of 19 of the 21 totara provenances originally sent to Christchurch from the Forest Research Institute, Rotorua. Further analysis of his data from Rangiora nursery and the glasshouse shows his results to correspond with those of the controlled environment trial.

Using individual seedling measurements, final seedling size in both environments was correlated ($p=0.05$) with annual

temperature. In the glasshouse, final seedling size was also correlated with annual precipitation (p=0.0001). Relative growth rate, calculated as: [ln(final size) - ln (initial size)]/56 days, was not correlated with any environmental or geographic variables in the Rangiora seedlings. This measure was significantly correlated with climate for the glasshouse seedlings, however it was shown that relative growth rate was correlated with plant size in these seedlings. When the effect of initial plant size on relative growth rate was removed by covariance analysis and the adjusted relative growth rates were compared with environmental and geographic variables, no significant correlations could be found.

DISCUSSION

Variation in Growth and its Correlation With Climate

Variation within a species is likely to occur when various segments of the population are subjected to quantitatively or qualitatively differing selection pressures. Species whose ranges parallel an environmental gradient are apt to be under quantitatively differing selection pressures, and these may create provenances well adapted to their location of origin.

While New Zealand has a relatively temperate climate, environmental gradients are strong enough to have created significantly different provenances of silver, black and mountain beech (Wilcox and Ledgard, 1983). The growth of these species is negatively correlated with their elevation of origin and, to some extent, with latitude; thus the slowest growing trees come from high altitude or southern locations. Ecotypic differentiation was also clearly indicated in manuka, with variation in leaf size and shape, basal area and height being correlated with environmental parameters such as latitude, altitude and distance from the coast (Ronghua et al., 1984).

The totara in the experiment under discussion did not show any clear pattern of variation. There were significant differences between provenances, both in total growth and preference of temperature regime, which were supported by observations of height growth in field and glasshouse conditions prior to the trial. The inter-provenance differences in growth rate however, were not well correlated with climatic or geographic variables. While absolute growth in total weight was correlated with latitude, days of ground frost, and mean temperatures when the Peel Forest provenance was removed, percentage growth was not correlated with anything. This means that, for the change in weight of totara seedlings as a percentage of their starting weights, there were no significant correlations of growth with environment.

A Comparison With Other Research on Variation in Totara Provenances

Although relative or percentage growth rates of the totara provenances in the controlled environment trial and Slui's (1988) nursery and glasshouse experiments could not be shown to correlate with their climates of origin under any conditions, the fact remains that from one year after sowing onwards, seedlings of provenances from higher, cooler and wetter locations were larger than those from lower, warmer or drier areas. Because these seedlings were larger, they grew more in absolute terms, but at a slower rate, over the course of the experiments; and this resulted in the negative correlation of absolute growth with the temperature of origin in the controlled environment trial, and the positive correlations of percentage growth with temperature and rainfall of origin in Slui's (1988) Rangiora and glasshouse experiments.

No correlation could be found between seed length and seedling size, thus the differences in first year growth were

probably not a result of the amount of stored carbohydrate available to the newly germinated seedlings. There was however, a large difference in the rate of germination among and within provenances, with some seeds taking almost one year to germinate. Bergin and Ecroyd (1987) state that the height differences between provenances in October, 1986 were more likely due to different times of germination than to differences in growth rate. Thus it is possible that seeds from provenances of cooler, moister regions germinate more rapidly than those from other areas which gives them a long term advantage in growth.

As the height of the seedlings in October, 1986 was highly correlated with their size in February, 1988 ($p=0.002$), these initial differences in germination are the most likely explanation for the lasting correlations of provenance size with climate. Other possible factors may be that the provenances from cooler, wetter areas have a longer growing season when conditions are favourable, thus gaining in size on their counterparts from warmer, drier areas. The Rangiora and glasshouse provenances in Slui's (1988) study also sustained serious frost damage in their first winter in Christchurch, and it is possible than the provenances from cooler areas were more frost tolerant and therefore were not reduced in size as much as those from milder climates. Thus while provenance differences may occur in germination rate, length of growing season or frost tolerance, no adaptation to current climates was apparent in the growth rates of the totara seedlings.

The correlation of the differences in height growth between the 'cool' and 'warm' C.E. cabinets with elevation may reflect provenance adaptation to temperature. In this case, provenances from higher elevations did not grow as tall in the warmer temperature as did provenances from lower elevations. While all provenances had similar height growth in the 21^{o}C regime, and all decreased to some extent in the

27°C regime, the high elevation provenances were much more adversely affected than the others.

Slui (1988) also found percentage height growth in the first growing season of seedlings planted at Rangiora to be positively correlated with the temperature of origin. Again, this may be a result of the seedlings from cooler areas germinating earlier and therefore being taller by the beginning of the first growing season, but it is also possible that seedling height growth may be under some selection pressure.

Wilcox and Ledgard (1983) found that the height growth of all species of beech was greater in a cooler environment; but they thought this might be due to delayed lining out of the seedlings in the warmer environment. The trend of decreasing height growth in provenances from higher altitudes has been documented in western larch (Rehfeldt, 1982), big tree (Guinon et al., 1982), Douglas-fir (Rehfeldt, 1983) and lodgepole pine (Rehfeldt and Wykoff, 1981).

Net Photosynthesis

Although the effect of temperature on photosynthetic rate was not significant in the provenance experiment, the inverse relationship of photosynthesis and growth is interesting. It appears that when leaf area is reduced, photosynthetic rate is increased; and whether this occurs in a cool or warm environment is provenance-dependent.

Research with balsam fir seedlings from a 1460 m elevational transect showed their photosynthetic temperature optimum to decrease with increasing elevation of seed source (Fryer and Ledig, 1972). The change in temperature optimum with elevation was similar to the adiabatic lapse rate, which suggested a precise adaptation to temperature through natural selection (Fryer and Ledig, 1972). In that species, genetic divergence had occurred despite a lack of physical barriers to gene exchange. In totara, no such correlation of optimum

temperature for photosynthesis and climate of seed source could be found even over widely separated sites. This might imply that environmental diversity in New Zealand is not sufficient to create local genotypes; or that selection pressures on totara have been for attributes other than optimum photosynthetic rate.

Variations in Response to Selection Pressures

While variation in optimum temperatures for growth appears to be present in the totara provenances, the variation does not seem to be linked to any geographic or climatic variables. This fact might support the theory that selection pressures have not acted significantly upon growth rate over the past few million years. Even in the absence of selection pressures, some genetic drift is to be expected, and, by chance, some populations could have altered to grow optimally at cooler temperatures. Most provenances, however, have maintained their adaptation to warm temperatures, or do not show a significant difference.

If selection pressures of the recent past have not favoured growth rate, they may have been acting upon some other traits. One possibility is that selection has been for frost resistance, and this will be discussed later in the thesis. It also appears that there may have been some selection pressure on height growth as all provenances, particularly those from cooler areas, achieved maximum leading shoot extension at lower temperatures than those which maximized total growth.

CHAPTER VI

AN INVESTIGATION OF GENETIC VARIATION IN RIMU, KAHIKATEA, TOTARA AND RELATED SPECIES USING ISOZYME ANALYSIS

INTRODUCTION

The results from the totara provenance experiment indicate that genetic variation does occur within this species. It is difficult to quantify this variation through experiments with seedlings however, because the interactions of genotypes and environments tend to obscure genetic differences.

The biochemical method of isozyme analysis is a powerful tool in the study of the genetic structure of plant or animal populations. In this study it was used, through the technique of starch gel electrophoresis, to obtain a general picture of the degree of genetic variation within and between populations of rimu, kahikatea, totara and some related species, without the complicating effects of environmental variation. It was hoped that a knowledge of the genetic structure of rimu, kahikatea and totara would be of value in explaining their ecological distribution.

Enzymes are generally the product of individual genes. The linear sequence of amino acids in an enzyme corresponds with the linear sequence of nucleotides in the DNA that produced it; thus an enzyme provides information on the gene from which it arose (Roughgarden, 1979). Mutations in the structural gene will result in the substitution of amino acids in the enzyme, and this may change its net charge and/or the configuration of the amino acid, altering the structure of the enzyme.

Electrophoresis is a process whereby chemicals, such as enzymes, are induced to migrate through a starch gel by

the application of an electric current. Different enzymes, or varying forms of the same enzyme (isozymes), migrate at different rates according to their electric charge, configuration, the current applied, and the pH of the gel and buffer solutions. Staining the gel for one enzyme allows visualization of isozyme migration, and measurements of this movement can be used to calculate the allelic variation in the sample.

Considerations of the genetic code and of the electrical properties of amino acids suggest that only 27.5 percent of all amino acid substitutions are detectable by gel electrophoresis (Shaw, 1965). When the variation in enzyme configuration detected by molecular sieving in the gel is accounted for however, it is estimated that 40 percent of all amino acid substitutions may be detectable by electrophoresis (Ramshaw et al., 1979). Thus, predictions of the genetic variation of populations by this method are likely to underestimate the actual variation by approximately one half.

MATERIALS AND METHODS

Enzyme source material varies widely, and the enzymes present often vary with the source material. For coniferous trees, enzymes can be extracted from needles, roots, bark, buds and seeds. The megagametophyte of the coniferous seed is a preferred material for analysis as it is haploid and therefore allows direct analysis of genetically determined enzyme mobility differences (Yeh and O'Malley, 1980). It is this tissue that was used in the study of genetic variation of all species except kahikatea. No enzymes could be clearly resolved from the kahikatea megagametophyte; therefore the germinating embryo was used as source material.

Seed Origin

Bulked collections of rimu seed were obtained from eight locations on the South Island. The collections varied in the number of trees from which seed was taken, but it was assumed that collections contained equal numbers of seed from each parent tree. The minimum number of seeds selected for analysis per population was six times the number of trees sampled. Six seeds per tree were chosen because, if the collections were not bulked, the analysis of haploid tissue from six seeds would identify a heterozygous locus in an individual with a probability of $[1 - (0.5)^5]$ or 0.97. Unfortunately, much of the rimu seed obtained for the study was inviable, therefore only five populations provided an adequate number of seeds for reliable analysis.

Individual-tree seed collections were made for five populations of kahikatea from the North and South Islands. As diploid embryo tissue was used as source material for kahikatea, only one embryo per tree was analyzed in all collections. Seed from the Tahuna and Hamilton populations was bulked. It was assumed that these two collections contained equal numbers of seed from each parent tree; therefore the number of diploid embryos sampled equalled the number of trees from which the seed was collected.

Seed was only available from two populations for three of the four totara species, however seed was obtained for five snow totara populations. Individual-tree seed collections from the North and South Islands were made for these species. Eight individuals provided the seed for the population of pygmy pine, and in all these species, the haploid megagametophytes of eight seeds per tree were analyzed, identifying a heterozygous locus in each individual with a probability of 0.99.

Details of the origins of the seed, the number of maternal parents in each sample population, and the numbers of seed analyzed for all species are presented in Table 27.

Table 27: Details of sample populations for isozyme analysis (see Appendix B for further details of seed origin)

Population	Elev. (m)	Lat.	No. of Maternal Parents	No. of Seed Analyzed	Annual Temp. (°C)	Annual Precip. (mm)	Days of Frost
RIMU							
1. Oparara	210	41° 07′	9	135	11.2	2200	20
2. Ahaura	150	42° 24′	5	60	11.1	1900	52
3. Hochstetter	260	42° 26′	10	15	10.7	1900	60
4. Hokitika	30	42° 48′	10	115	11.6	2783	16
5. Saltwater	75	43° 08′	11	65	11.0	3789	65
6. Jackson Bay	45	44° 03′	5	55	10.9	4663	4
7. Cascade S.F.	-	- -	5	20	11.3	3455	7
8. Waitutu S.F.	45	46° 14′	20	30	10.2	997	44
KAHIKATEA							
1. Warkworth	60	36° 23′	4	4	14.6	1514	3
2. Tahuna	30	37° 30′	10	10	14.4	1178	5
3. Hamilton	45	37° 47′	10	10	13.3	1201	26
4. Pureora	550	38° 31′	6	6	10.5	1804	48
5. Kumara	105	42° 41′	9	9	11.3	1892	54
6. S. Westland	120	43° 26′	7	7	10.9	4540	25
7. Christchurch	10	43° 32′	21	21	11.6	666	36
TOTARA							
1. Warkworth	30	36° 23′	13	104	14.6	1514	3
2. Owhango	440	39° 00′	9	72	12.0	1586	38
TOTARA VAR. WAIHOENSIS							
1. Lafontaine R.	45	43° 07′	12	96	11.1	3789	65
2. Lk. Matheson	70	43° 27′	12	96	10.9	4540	25
HALL'S TOTARA							
1. Whakapapa	595	39° 06′	4	32	12.0	1202	65
2. Ohakune	975	39° 21′	7	56	7.5	1600	70
SNOW TOTARA							
1. Whakapapa	1250	39° 12′	17	136	6.2	2838	90
2. Jack's Pass	915	42° 28′	5	40	8.1	910	128
3. Temple Basin	950	42° 54′	12	96	7.9	5042	110
4. Hooker Val.	715	43° 43′	4	32	8.4	4098	95
5. Rob Roy Val.	1200	44° 28′	7	56	7.7	800	105
PYGMY PINE							
1. Whakapapa	1250	39° 12′	8	64	6.2	2838	90

Electrophoretic Procedures

Rimu seeds were germinated under a temperature regime of 30°/20°C for 12 hr/12 hr until 2 mm of radicle had emerged. The seeds were germinated in order to take advantage of the subsequent increased enzyme activity (Toole et al., 1956). The seeds of the totara species and pygmy pine were removed from cold storage and held at 18°C on moist filter paper for one week before preparation in order to increase enzyme activity. Kahikatea seeds were germinated at a constant 18°C until 5 mm of radicle had emerged. The megagametophytes or embryos, depending on species, were removed and individually homogenized in 1 ml auto-analysis cups with two drops of extraction buffer.

The auto-analysis cups and their contents were frozen to break down cell walls and to preserve enzyme activity. Immediately before electrophoresis, the auto-analysis cups were thawed and their contents absorbed onto two 15 mm X 1.5 mm filter paper wicks (Whatman No. 3) which were then inserted along a cut 4 cm from the edge of two different 12.5 or 13 percent (w/v) starch gels.

The gels, 8 cm(w) X 23 cm(l) X 1 cm(d) would accommodate up to 90 samples along the longitudinal axis. Wicks soaked in tissue extract from a homozygous line of Pisum sativum were inserted between every ten samples as a standard for comparison between gels. Wicks soaked in red food colour were inserted at the ends of the gel to monitor the migration of the proteins.

After loading, the gels were placed over buffer trays either in a cold (4°C) room or under an ice pack to prevent heating and denaturing of the proteins, and the appropriate current applied. Once the food colour tracking dye had migrated 10 mm (1/2 hr), all wicks were removed as the protein they contained had migrated into the gel. The gels were then covered in plastic wrap to keep them from drying, and the electrophoresis continued.

When electrophoresis was completed (4 to 5 hr), each starch gel was sliced horizontally into eight, 1 mm thick, strips. Each gel slice was placed in a tray containing a specific enzyme stain solution. Details of the recipes for extraction, gel and electrode buffers, voltages and enzyme stain solutions are listed in Appendix U.

Analysis of Isozyme Variation

Eighteen enzyme systems were studied in various combinations in the different species: AAT, ACON, ACP, ADH, DIA, GDH, G6P, IDH, LAP, MDH, ME, PEP, 6PG, PGI, PGM, SDH, SKDH, and SOD (for official names of the enzymes systems, see Appendix U).

For each species, when an enzyme was produced by multiple loci, the loci were identified by the symbol of the enzyme and a hyphenated numeral. The most anodal loci was numbered 1, the next 2, and so on. Within each locus, the allozymes were designated by the distance they migrated as a proportion of the distance the most common allozyme in the species (labelled 100) migrated. The frequencies of alleles at each locus were calculated, and from the allelic frequencies, several measures of genetic variation were computed.

For each locus, the expected heterozygosity (He) for a population in Hardy-Weinberg equilibrium was calculated with Nei and Roychoudhury's (1974) formula:

$$He = (1 - \Sigma x_i^2) \qquad \text{where } x_i = \text{frequency of the } i^{th} \text{ allele in the sample population}$$

This measure was averaged over all loci for each population, but the average is only likely to represent the heterozygosity of the entire population if the sample population contained a relatively large number of individuals. Sample populations of 36 individuals are generally considered to be the minimum size (B.P. Dancik, pers. comm.), and with such a population, one can be 95 percent sure of sampling any alleles present at a 5 percent

frequency or greater in the entire population. In this study, all sample populations were smaller than the minimum size, therefore in the calculations of expected heterozygosity, a correction for small sample size was included (Nei, 1978). These calculations, plus a calculation of the standard error of expected heterozygosity (Nei and Roychoudhury, 1974), were performed with the computer program of Dowling and Moore (1984). Average expected heterozygosities, weighted by sample population size, were calculated for each locus in each species.

The proportion of polymorphic loci and the effective number of alleles (Lundkvist, 1979) averaged geometrically over all loci were also calculated for each population. The mean, weighted by sample population size, was then calculated for each of these statistics for each species.

To investigate the partitioning of total population diversity (Ht) into its 'within' (Hs) and 'between' (Dst) components, Nei's (1973) relative measure of gene diversity (Gst) was calculated.

$$Gst = Dst / Ht$$

and

$$Ht = Hs + Dst$$

where Hs = weighted average of expected heterozygosities at all loci in all sub-populations

Ht = heterozygosity calculated with the mean allele frequencies.

A value of one for Gst means that all of the genetic diversity at that locus occurs among sample populations, that is Hs=0 and there is no variation within populations. A value of zero for Gst indicates that Dst=0 and there is no variation or differentiation between populations at that locus.

For rimu, kahikatea and snow totara, the species with more than two sample populations, correlation analyses were run between the corrected expected heterozygosities of the

various loci, and several climatic and geographic variables. This was intended to indicate if the variable loci were selectively important.

Nei's (1972) measure of genetic identity (I), genetic distance (D), and their standard errors (Nei and Roychoudhury, 1974) were also calculated with the computer program of Dowling and Moore (1984). These variables represent the 'relatedness' of the various populations.

$I = Ixy / \sqrt{(IxIy)}$ where Ixy, Ix and Iy are the arithmetic means over all loci of $x_i y_i$, x_i^2 and y_i^2 respectively.

and the variables x_i and y_i are the frequencies of the i^{th} allele in populations X and Y

The genetic distance is calculated by: $D = -\ln I$ and measures the genetic differentiation among populations.

A value of one for D means that the populations have no alleles in common at a locus, and a value of zero indicates that the populations have the same alleles at identical frequencies.

To visualize the order of population divergence more easily, dendrograms can be created from genetic distance calculations through agglomerative clustering (Sneath and Sokal, 1973). This was done for all species.

Finally, to examine the degree to which observed heterozygosity deviates from that expected under random mating, Wright's fixation index 'F' (Wright, 1922) was calculated.

$F = 1 - (Ho/He)$ where Ho = observed heterozygosity
He = expected heterozygosity

This measure was only calculated for the largest sample population in each species, and so represents the

level of inbreeding in that population, rather than in the species as whole. No value could be obtained for rimu since the seed collections had been bulked.

RESULTS

As population samples were smaller than desired, and band patterns were not completely clear for some enzymes, results from this study should be regarded as preliminary.

Measures of Variation Within Species

(i) Rimu. Twenty-five loci were resolved with sufficient clarity and consistency to be included in the results. Twenty of these loci were monomorphic: AAT-1, ACP-2, ACP-3, ACP-4, ACP-5, ADH-1, GDH-1, G6P-1, IDH-1, LAP-1, MDH-2, ME-2, PEP-1, PEP-2, PEP-3, 6PG-1, 6PG-2, PGI-1, PGI-2,and SDH-1. Table 28 presents the frequencies of alleles for the five heterozygous loci in the various populations. The table also lists: for each polymorphic locus in each population, the proportions, corrected for small sample sizes, of individuals that could be expected to be heterozygous if the populations are in Hardy-Weinberg equilibrium; the mean He for each sample population and its standard error; and the gene diversity index for each polymorphic locus.

ME-1 was the most heterozygous locus with an average He, weighted by population size, of 49.1 percent. MDH-1 was the next most heterozygous loci at 30.1 percent. The other three polymorphic loci, AAT-2, ACON-1 and ACP-1 had weighted average He's of 24.4, 22.1 and 16.7 percent respectively.

Table 28: Allelic variation of 5 variable loci, their expected heterozygosities (He), the weighted mean He and its standard error (S.E.) for each population, and the gene diversity indices in 5 sample populations of rimu (Populations 3, 7 and 8 were represented by too few seeds for reliable analysis)

Locus	Allele	Populations 1	2	3	4	5	6	7	8	Gst
AAT-2	133	.17	.02	.09	18	11	16	?	.07	
	100	.83	.98	.91	.82	.89	.84	?	.93	
	He	.299	.044		.311	.205	.299			
										.0316
ACON-1	100	.81	.86	?	.90	.89	.96	.94	.88	
	66	.19	.14	?	.10	.11	.04	.06	.12	
	He	.326	.267		.189	.205	.085			
										.0217
ACP-1	100	.89	?	.79	.92	.95	.86	1.0	.93	
	null	.11	?	.21	.08	.05	.14	0.0	.07	
	He	.207	?		.155	.099	.267			
										.0457
MDH-1	100	.87	.89	.69	.64	.92	.72	.62	.73	
	75	.13	.11	.31	.36	.08	.28	.38	.27	
	He	.239	.217		.485	.154	.448			
										.0658
ME-1	133	.33	?	?	.38	.46	.29	.38	.21	
	100	.67	?	?	.62	.54	.71	.62	.79	
	He	.468	?	?	.496	.520	.458			
										.0273
Mean	He	.062	.029		.065	.047	.062			
S.E.		.026	?		.030	.023	.028			
(25 loci)										.0320

(ii) Kahikatea. Twenty-three loci were scored reliably in kahikatea. Nineteen of these loci were monomorphic: AAT-2, AAT-3, AAT-4, ACP-1, ACP-2, DIA-1, GDH-1, G6P-1, LAP-1, MDH-1, MDH-2, PEP-1, PEP-2, PEP-3, 6PG-1, PGI-1, PGI-2, PGM-1 and SOD-1. No activity for ACON, ADH, ME or SDH could be detected in this species. These enzymes were

replaced by DIA, PGM, and SOD which could be scored from gels stained for ME. Table 29 presents the allele frequencies and statistics of variation for the four polymorphic loci in the kahikatea sample populations.

MDH-3 was the most heterozygous locus in kahikatea with a weighted average He of 35.8 percent. IDH-1 and AAT-1 were similar, with weighted average He's of 25.1 and 23.9 percent respectively. PGI-3 was only variable in two sample populations, and therefore had a weighted average He of only 4.2 percent.

Table 29: Allelic variation of 4 variable loci, their He's, the weighted mean He and its S.E. for each population, and the gene diversity indices in 7 sample populations of kahikatea

Locus	Allele	Populations							Gst
		1	2	3	4	5	6	7	
AAT-1	109	0	0	0	0	.05	.14	.05	
	100	.63	.91	.73	1.0	.95	.86	.86	
	92	.37	.09	.27	0	0	0	.09	
	He	.533	.172	.405	0	.101	.259	.256	
									.0448
IDH-1	100	.50	1.0	.89	1.0	.89	.86	.71	
	67	.50	0	.11	0	.11	.14	.29	
	He	.571	0	.201	0	.207	.259	.422	
									.0951
MDH-3	143	0	0	.33	.25	.22	.43	.38	
	100	1.0	1.0	.67	.75	.78	.57	.62	
	He	0	0	.455	.391	.363	.528	.483	
									.0808
PGI-3	100	1.0	1.0	1.0	1.0	.89	.93	1.0	
	87	0	0	0	0	.11	.07	0	
	He	0	0	0	0	.207	.140	0	
									.0162
Mean	He	.048	.007	.046	.017	.038	.052	.050	
S.E.		.033	.007	.001	.017	.001	.001	.029	
(23 loci)									.0675

(iii) Totara Species. Twenty-four loci were scored consistently in totara, totara var. waihoensis and Hall's totara. Sixteen of these loci were monomorphic in totara, fourteen in Hall's totara and thirteen in totara var. waihoensis. AAT-3, ACP-1, DIA-2, MDH-2, PEP-2, PEP-3, 6PG-2, PGI-1, PGM-1 and SDH-1 were monomorphic in all three species. SKDH-1 was monomorphic only in totara and GDH-1 was monomorphic only in Hall's totara. AAT-1 and IDH-1 were monomorphic in totara and totara var. waihoensis, while MDH-1 and 6PG-1 were monomorphic in totara and Hall's totara. No activity could be detected for ACON, ADH, LAP or SOD in these species. These enzymes were replaced by DIA, PGM and SKDH. Table 30 lists the allele frequencies for polymorphic loci in these species, plus the other calculated variables included in the tables for rimu and kahikatea.

In totara, DIA-1 was the most heterozygous locus with a weighted average He of 55 percent. G6P-1 was also highly heterozygous with a weighted average He of 52.9 percent. AAT-4 was highly polymorphic in the Warkworth sample population, but was monomorphic in the Owhango sample population, therefore the weighted average He at this locus was 23.2 percent. The least variable locus was GDH-1, with an He of 4.9 percent, as it was variable only in the Owhango sample population. The weighted average He of the other variable loci ranged from 13.9 to 45.1 percent.

In totara var. waihoensis, G6P-1 was the most heterozygous locus with an average He of 48.3 percent. As in totara, AAT-4 and GDH-1 were polymorphic in only one of the two populations, but the level of heterozygosity was low thus their average He's were 4 percent. DIA-1 was polymorphic only in the Lake Matheson sample population, but in this population it had a relatively large He therefore the average He was 17.3 percent. Other loci had average He's ranging from 18.7 to 35 percent.

For Hall's totara, DIA-1 and G6P-1 were the most heterozygous loci, with weighted average He's of 50 and 49 percent respectively. The least heterozygous locus, averaging 8.9 percent heterozygosity, was AAT-1 as it had a low degree of polymorphism in the Ohakune population only. AAT-4 and IDH-1 were highly polymorphic in one population only, thus both loci had weighted average He's around 16 percent. Other loci had weighted average He's between 30 and 40 percent.

Twenty-one loci were resolved reliably in snow totara, eleven of which were monomorphic: AAT-1, AAT-3, ME-2, PEP-1, PEP-2, PEP-3, 6PG-1, 6PG-2, PGI-1, PGI-2 and PGM-1. No activity was detected for ACON, ACP, ADH, LAP, SDH or SKDH; but SOD could be scored from gels stained for ACP. Table 31 lists the allele frequencies, individual locus and mean He's and the standard errors, and Gst values for polymorphic loci in snow totara.

SOD-1 was the most variable locus in snow totara with a weighted average He of 47.8 percent. This locus and G6P-1 were the only polymorphic loci that were heterozygous in all populations. The least variable loci were DIA-2, MDH-1 and GDH-1 with weighted average He's of 4.0, 4.4 and 6.4 percent respectively. Other loci had weighted average He's ranging from 8.3 to 38.0 percent.

An analysis of seed from the eight individuals of pygmy pine collected with the Whakapapa snow totara seed showed twelve loci could be scored clearly. All were monomorphic: AAT-1, AAT-2, DIA-1, GDH-1, G6P-1, IDH-1, IDH-2, MDH-2, ME-1, ME-2, PEP-3 and SDH-1.

Table 30: Allelic variation of 13 variable loci, their He's, the weighted mean He and its S.E. for each population, and the gene diversity indices in sample populations of totara, totara var. waihoensis and Hall's totara

Locus	Allele	Populations								
		Totara			T. var.waihoensis			Hall's totara		
		1	2	Gst	1	2	Gst	1	2	Gst
AAT-1	115	0	0		0	0		0	.07	
	100	1.0	1.0		1.0	1.0		1.0	.93	
	He	0	0		0	0		0	.140	
				0			0			.0148
AAT-2	133	.08	.06		.08	.21		.12	.21	
	100	.92	.94		.92	.79		.88	.79	
	He	.153	.119		.153	.346		.241	.357	
				.0037			.0339			.0117
AAT-4	100	.77	1.0		1.0	.96		1.0	.86	
	73	.08	0		0	0		0	.14	
	null	.15	0		0	.04		0	0	
	He	.393	0		0	.080		0	.259	
				.1054			.0204			.0555
DIA-1	110	.31	.28		0	.21		.25	.07	
	100	.58	.66		1.0	.79		.50	.93	
	80	.11	.06		0	0		.25	0	
	He	.578	.511		0	.346		.714	.140	
				.0037			.1173			.1826
GDH-1	100	1.0	.94		.96	1.0		1.0	1.0	
	90	0	.06		.04	0		0	0	
	He	0	.119		.080	0		0	0	
				.0563			.0204			0
G6P-1	141	0	.06		.04	.10		0	0	
	120	.46	.33		.67	.70		.50	.71	
	100	.54	.61		.29	.20		.50	.29	
	He	.517	.546		.486	.480		.571	.443	
				.0131			.0066			.0435
IDH-1	100	1.0	1.0		1.0	1.0		.75	1.0	
	75	0	0		0	0		.25	0	
	He	0	0		0	0		.429	0	
				0			0			.1673

Table 30: Continued

Locus	Allele	Populations								
		Totara			T. var.waihoensis			Hall's totara		
		1	2	Gst	1	2	Gst	1	2	Gst
MDH-1	100	1.0	1.0		.79	.92		1.0	1.0	
	63	0	0		.21	.08		0	0	
	He	0	0		.346	.154		0	0	
				0			.0339			0
PEP-1	118	.08	.28		.12	.08		.12	.29	
	100	.92	.72		.88	.92		.88	.71	
	He	.153	.427		.220	.154		.241	.443	
				.0721			.0044			.0435
6PG-1	106	0	0		.25	.08		0	0	
	100	1.0	1.0		.75	.92		1.0	1.0	
	He	0	0		.391	.154		0	0	
				0			.0526			0
PGI-2	108	0	0		0	0		0	.14	
	100	.85	.72		.96	.83		.75	.86	
	92	.15	.28		.04	.17		.25	0	
	He	.265	.427		.080	.294		.429	.259	
				.0246			.0449			.0700
SDH-2	114	.44	.22		.25	.18		.25	.21	
	100	.56	.78		.75	.82		.75	.79	
	He	.512	.363		.391	.308		.429	.357	
				.0514			.0071			.0003
SKDH-1	100	0	1.0		.46	.92		.75	.86	
	57	1.0	0		.54	.08		.25	.14	
	He	0	0		.518	.154		.429	.259	
				0			.2473			.0190
Mean	He	.107	.105		.111	.103		.145	.111	
S.E.		.039	.039		.036	.030		.046	.033	
(24 loci)				.0350			.0664			.0606

Table 31: Allelic variation of 10 variable loci, their He's, the weighted mean He and its S.E. for each population, and the gene diversity indices in 5 sample populations of snow totara

Locus	Allele	Populations					Gst
		1	2	3	4	5	
AAT-2	133	.06	0	.08	.13	0	
	100	.94	1.0	.92	.87	1.0	
	He	.116	0	.154	.258	0	
							.0350
AAT-4	100	.79	1.0	.88	1.0	.75	
	73	.06	0	.04	0	0	
	null	.15	0	.08	0	.25	
	He	.360	0	.227	0	.404	
							.0522
DIA-1	110	.23	.10	.13	0	0	
	100	.77	.90	.87	1.0	1.0	
	He	.365	.200	.236	0	0	
							.0716
DIA-2	125	0	.20	0	0	0	
	100	1.0	.80	1.0	1.0	1.0	
	He	0	.356	0	0	0	
							.1728
GDH-1	100	.91	1.0	1.0	1.0	1.0	
	90	.09	0	0	0	0	
	He	.169	0	0	0	0	
							.0577
G6P-1	120	.68	.60	.87	.50	.87	
	100	.32	.40	.13	.50	.13	
	He	.448	.533	.236	.571	.244	
							.0817
IDH-1	125	0	0	.04	0	0	
	100	1.0	.90	.88	1.0	1.0	
	75	0	.10	.08	0	0	
	He	0	.200	.227	0	0	
							.0384
MDH-1	100	1.0	1.0	.96	.87	1.0	
	63	0	0	.04	.13	0	
	He	0	0	.080	.258	0	
							.0967

Table 31: Continued

Locus	Allele	Populations 1	2	3	4	5	Gst
MDH-2	278	0	.10	.29	.37	.25	
	100	1.0	.90	.71	.63	.75	
	He	0	.200	.430	.533	.404	
							.1459
SOD-1	110	.06	.10	.04	.50	.14	
	100	.56	.90	.75	.50	.65	
	90	.38	0	.21	0	.21	
	He	.555	.200	.409	.571	.553	
							.0945
Mean	He	.096	.080	.095	.104	.076	
S.E.		.038	.032	.031	.045	.037	
(21 loci)							.0854

Measures of Variation Between Species

The locus and allele numbering systems were devised separately for the three genera. A particular, numbered isozyme therefore, is not necessarily the same isozyme in two different genera but is the same isozyme within a genus.

The most common alleles (100) at every locus in rimu and kahikatea were most common with respect to their frequency in the populations, but also with respect to the number of populations containing them. This indicates a high degree of similarity in the allelic composition over all loci of all populations in these two species. This generalization was also true for most loci in the totara species. For G6P-1 however, the '100' allele was most common only in totara, while the '120' allele was predominant in the other three totara species. In the Lafontaine River sample population of totara var. waihoensis, the frequency of the SKDH-1 '57' allele was greater than that of the '100' allele which was most common in all other populations.

Overall mean expected heterozygosities, weighted by sample population size, for each species showed kahikatea and rimu to be the least heterozygous, with average He's of 3.87

and 5.83 percent respectively. The totara species were more heterozygous and their average He's were as follows: snow totara 9.17 percent, totara 10.61 percent, totara var. waihoensis 10.7 percent, and Hall's totara 12.33 percent. An analysis of variance of the mean He's of each population showed a significant difference ($p=0.001$) in variability between species. Scheffe's test of means ($p=0.05$) showed kahikatea to be significantly less variable than all other species except rimu. Rimu was significantly less variable than all the totara species except snow totara, while the levels of variability within the totara species were not significantly different from one another.

For each population of each species, the percentage of polymorphic loci, effective number of alleles and their weighted means are presented in Table 32. This table again displays the high degree of uniformity among populations of kahikatea and rimu. The kahikatea populations were polymorphic for between 4 and 17 percent of their loci using the 95 percent criterion for polymorphism, while the percentage of polymorphic loci ranged from 15 to 20 percent in rimu.

There were much higher percentages of polymorphic loci in the totara species, ranging from 19 to 42 percent. An analysis of variance of these figures showed there to be a significant difference ($p=0.001$) between species in the percentage of polymorphic loci. Scheffe's test of means ($p=0.05$) showed kahikatea to be significantly less variable than all other species except rimu. Rimu was not significantly different from the totara species except for Hall's totara, and there were no significant differences in the percentages of polymorphic loci among the totara species.

The effective number of alleles per locus was close to one for all species, but again there were significant differences ($p=0.001$) between the species. The effective number of alleles was not significantly different in kahikatea and rimu using Scheffe's means test ($p=0.05$). Snow totara was significantly different from kahikatea but not from rimu or the rest of the totara species. The other

totara species did not differ significantly in their effective numbers of alleles, but were significantly different from kahikatea and rimu.

Table 32: Genetic variation in natural populations of rimu, kahikatea and totara species

Population	Species					
	Rimu	Kahikatea	Totara	Totara var. waihoensis	Hall's Totara	Snow Totara
% POLYMORPHIC LOCI[1]						
1.	20.0	8.7	29.2	37.5	30.0	28.6
2.	15.0	4.3	29.2	41.7	37.5	28.6
3.		13.0				38.1
4.	20.0	4.3				28.6
5.	16.0	17.4				19.1
6.	16.0	17.4				
7.		13.0				
Weighted Mean	18.2	10.2	29.2	39.6	34.8	29.6
EFFECTIVE NO. OF ALLELES						
1.	1.073	1.048	1.146	1.143	1.183	1.127
2.	1.057	1.008	1.137	1.125	1.132	1.091
3.		1.058				1.114
4.	1.082	1.021				1.128
5.	1.057	1.042				1.096
6.	1.071	1.061				
7.		1.065				
Weighted Mean	1.070	1.048	1.142	1.134	1.151	1.115

[1] Where the frequency of the most common allele is ≤ 0.95

The values of Gst in Tables 28 to 31 show a fair degree of variation between loci and between species. In rimu and totara, approximately three percent of the variation in the species was due to variation between populations. Average values of Gst were higher for the other species, and in Hall's totara, totara var. waihoensis, snow totara and kahikatea, between 6 and 8.5 percent of the genetic variation was between populations. Gst values for individual loci were

so variable that no significant differences between the species were evident.

Correlation of Variation With Geography and Climate

While the different indices of variation demonstrated significant differences between species, there was not enough variability within species to show any clinal trends. In spite of this, an attempt was made to correlate geographic and climatic variables with the heterozygosities of the polymorphic loci in rimu, kahikatea and snow totara (see Table 27 for geographic and climatic variables).

For rimu, expected heterozygosities at three of the five polymorphic loci showed some correlation ($p=0.10$) with geographic or climatic variables. AAT-2 was negatively correlated with days of frost ($p=0.01$) and showed a trend towards being correlated with latitude ($p=0.08$). ACP-1 tended towards correlation with precipitation ($p=0.09$), while MDH-1 was negatively correlated with elevation ($p=0.01$).

Kahikatea showed no significant correlation of heterozygosity and climatic or geographic variables at any locus.

For the five snow totara populations, heterozygosities of seven of the ten variable loci were well correlated with one or more environmental variables. AAT-2 showed a slight, positive correlation with precipitation ($p=0.06$) while AAT-4 was positively correlated with elevation ($p=0.05$). Expected heterozygosity at GDH-1 had a strong negative correlation with both latitude ($p=0.03$) and mean annual temperature ($p=0.01$). MDH-2 was positively correlated with latitude ($p=0.05$). DIA-1 showed a negative correlation with latitude ($p=0.03$), while both DIA-2 and SOD-1 were correlated with days of frost, DIA-2 positively ($p=0.01$) and SOD-1 negatively ($p=0.02$).

In both rimu and kahikatea, AAT was negatively correlated to some extent ($p=0.01$ and 0.13 respectively) with

days of frost. In rimu and snow totara, MDH-1 showed some negative correlated with elevation ($p=0.01$ and 0.10 respectively). Although they are not always strong, these and the other correlations may indicate that the variable loci are selectively important.

Population Differentiation

In spite of low heterozygosities, few polymorphic loci, and low inter-population gene differences in some species, Nei's (1972) genetic distance statistics were calculated for all species (Table 33). When the genetic distance values were corrected for the small sample sizes, many became negative which essentially meant there were no differences between the populations. For this reason, the genetic distances were recalculated without the correction for small sample sizes. These values were used for general comparisons within species. Dendrograms of population differentiation within species were derived from the genetic distance values. These figures indicate the degree of 'relatedness' of the populations.

For rimu, if all eight populations were included and average values estimated for missing alleles, the populations roughly split into northern and southern groups (Figure 15). The grouping was not perfect as the Saltwater population was grouped with northern populations, while the Hokitika population was grouped with southern populations contrary to their geographical positions. For kahikatea, the grouping appeared random therefore the dendrogram was not included.

Table 33: Genetic distance measures between populations of rimu, kahikatea and totara species without corrections for sample size

Populations	1	2	3	4	5	6	7
RIMU							
2	.0010						
3	.0023	.0025					
4	.0027	.0038	.0012				
5	.0014	.0011	.0039	.0038			
6	.0020	.0026	.0008	.0009	.0035		
7	.0041	.0041	.0022	.0054	.0043	.0017	
8	.0021	.0019	.0016	.0021	.0042	.0011	.0021
KAHIKATEA							
2	.0145						
3	.0123	.0066					
4	.0202	.0031	.0039				
5	.0151	.0033	.0038	.0011			
6	.0194	.0098	.0031	.0032	.0024		
7	.0116	.0101	.0026	.0050	.0034	.0016	

TOTARA SPECIES										
Totara		Totara var. waihoensis		Hall's Totara			Snow Totara			
1	2	1	2	1	2	1	2	3	4	
Totara										
2 .0065										
Totara var. waihoensis										
1 .0143	.0100									
2 .0221	.0198	.0073								
Hall's Totara										
1 .0164	.0145	.0071	.0110							
2 .0083	.0073	.0132	.0245	.0198						
Snow Totara										
1 .0180	.0126	.0105	.0113	.0132	.0160					
2 .0314	.0202	.0102	.0145	.0142	.0244	.0091				
3 .0241	.0202	.0165	.0143	.0184	.0264	.0095	.0104			
4 .0353	.0223	.0140	.0154	.0155	.0327	.0115	.0031	.0131		
5 .0167	.0070	.0070	.0116	.0098	.0155	.0070	.0085	.0155	.0090	

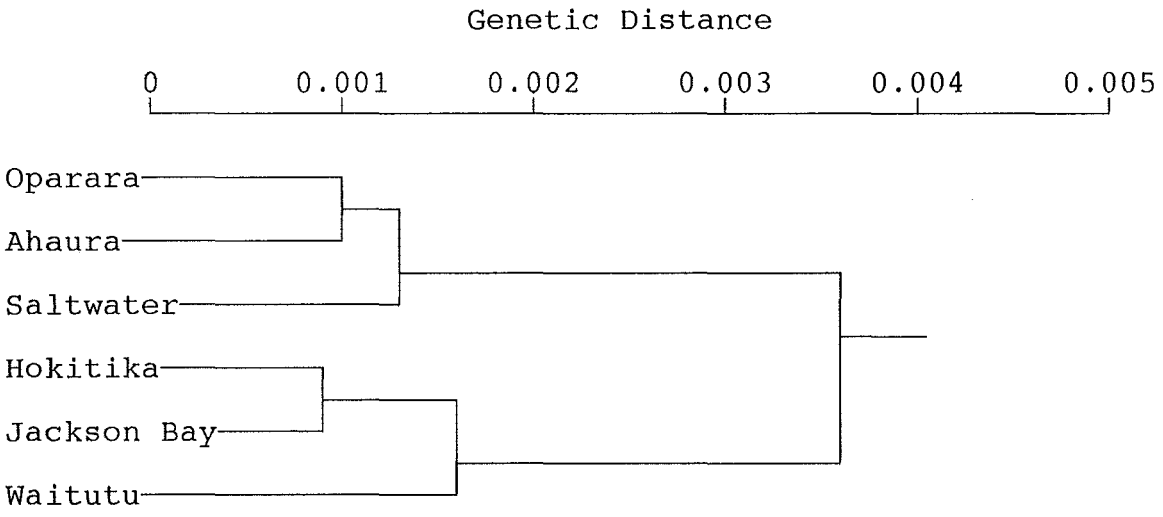

Figure 15: Plot of 'relatedness' of provenances of rimu

The analysis for the totara species was done using the common alleles between the four species (Figure 16). This clustering grouped the snow totara populations together, and these were most similar to the totara var. waihoensis populations. The two totara populations were grouped together as being distinct from the first two species, and the Hall's totara populations were split between the true totara and totara var. waihoensis groups.

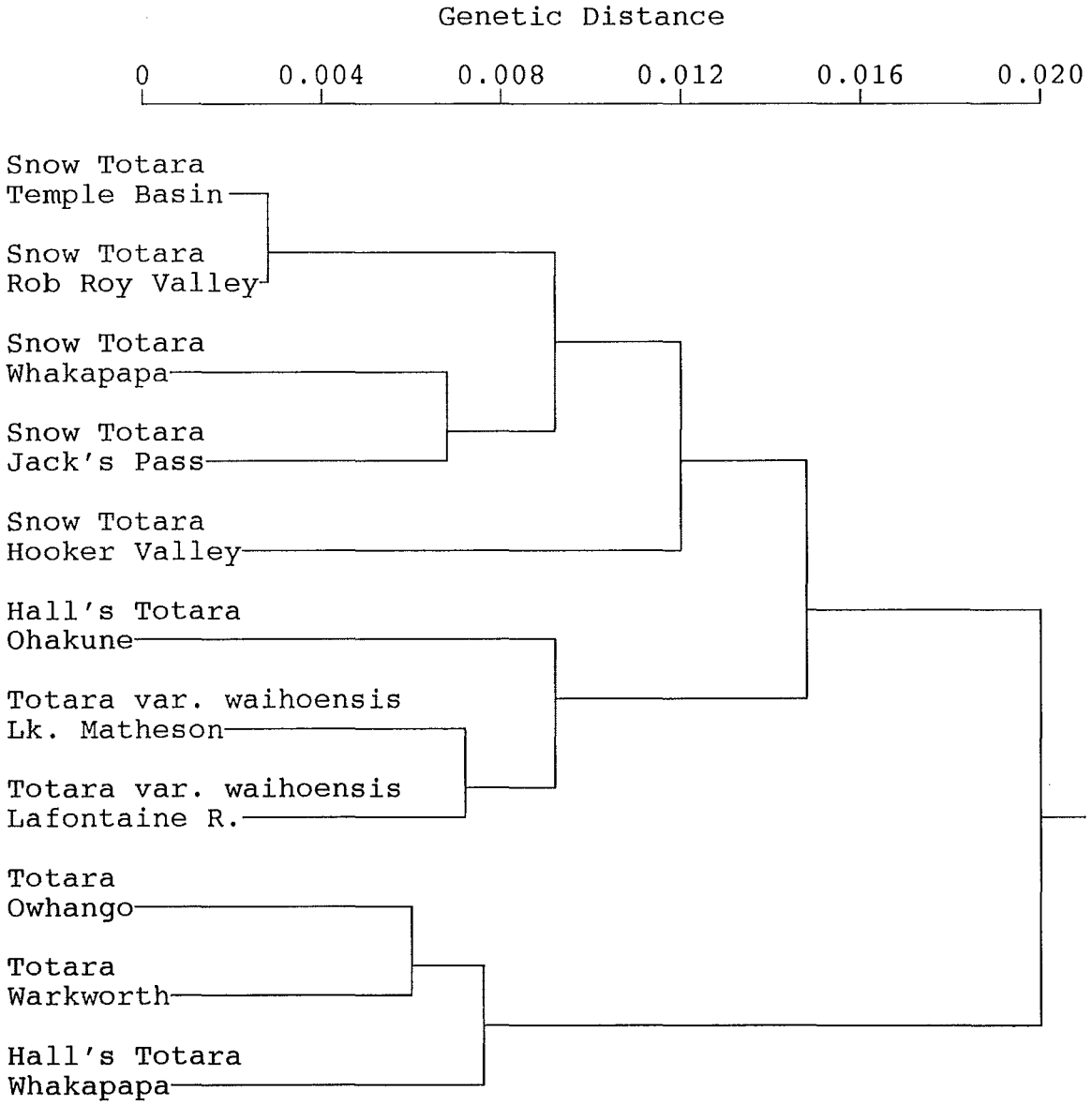

Figure 16: Plot of 'relatedness' of provenances of totara species

Species Differentiation

The genetic distance calculations including all seven species were computed using the fifteen loci common to all species and the actual numbers of individuals analyzed (Table 34). These calculations showed the genetic distances between the genera to be significant (p=0.05). The genetic distance

between pygmy pine and rimu was less significant (p=0.10) and the genetic distances between the totara species were no longer significant. The dendrogram created from the genetic distance figures (Figure 17) again displayed the similarity between the various species of Podocarpus. Rimu and pygmy pine were also grouped together, although they were much less closely related than the totara species. Kahikatea appeared to be more similar to the genus Podocarpus to which it once was assigned than to rimu and pygmy pine. Rimu and pygmy pine were only distantly related to the other species analyzed.

Table 34: Genetic distance measures between rimu, kahikatea and totara species

	Rimu	Pygmy Pine	Kahikatea	Totara	Totara var. waihoensis	Hall's Totara
Pygmy Pine	.1519*					
Kahikatea	.2337**	.3191**				
Totara	.2937**	.3806**	.1906**			
Totara v. waihoensis	.3061**	.3887**	.2007**	.0095		
Hall's Totara	.2928**	.3896**	.1936**	.0019	.0030	
Snow Totara	.3073**	.3997**	.2024**	.0122	.0061	.0053

* Genetic distance between species is significant (p=0.10)
** Genetic distance between species is significant (p=0.05)

The figures for genetic distance between totara populations are smaller in Figure 17 and Table 34 than in Figure 16 and Table 33 because fewer variable loci were used in the calculations. For the calculation including all seven species, there were only 15 loci common to all species, thus

the figures are different from those involving more loci. For this reason, and because the rates of mutation in podocarp species are unknown, there is little value in calculating times of divergence for the species from the genetic distances.

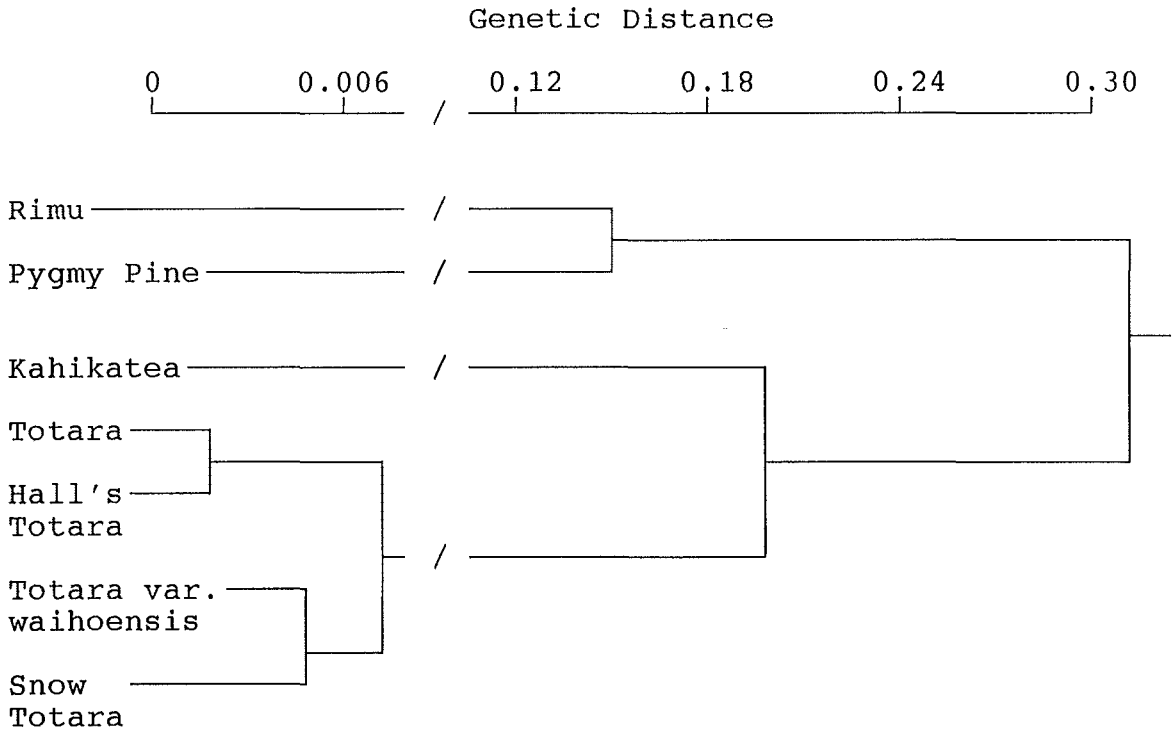

Figure 17: Plot of 'relatedness' of seven podocarp species

Fixation Indices

The fixation indices of the heterozygous loci in the largest populations of kahikatea and the totara species are presented in Table 35. In the Christchurch kahikatea population, a weighted average of the 'F' values over all loci indicated a 65.5 percent deficiency of heterozygotes relative to Hardy-Weinberg expectations. For two of the three variable loci, the deficiency was significantly different from the expected value ($p=0.01$), and the deficiency at the third locus was different from that expected with a probability of $p=0.12$.

The Whakapapa snow totara population had a 29.8 percent deficiency of heterozygotes on average, but the proportion of heterozygotes at any of the six variable loci was not significantly different from that expected under Hardy-Weinberg conditions. No significant differences from panmixia were shown for the nine variable loci in the Ohakune population of Hall's totara; although the average inbreeding coefficient was 24.1 percent.

Table 35: Fixation indices (F) of the variable loci in the largest populations of kahikatea and totara species

Locus	Kahikatea	Totara	Totara var. waihoensis	Hall's Totara	Snow Totara
AAT-1	0.619			-0.099	
AAT-2		-0.050	0.749	0.569	-0.042
AAT-4		0.187	-0.087	1.000	0.496
DIA-1		-0.385	0.246	-0.090	0.335
GDH-1					0.640
G6P-1		0.070	0.094	0.306	0.054
IDH-1	0.769*				
MDH-1			-0.130		
MDH-3	0.596*				
PEP-1		-0.047	-0.130	0.306	
6PG-1			-0.130		
PGI-2		-0.210	-0.183	-0.190	
SDH-2		0.746*	1.000*	0.569	
SKDH-1			-0.130	-0.190	
SOD-1					0.235

* Significantly different from panmixia (F=0) (p=0.05)

Of the seven variable loci in totara and the ten in totara var. waihoensis, only one locus showed a significant deficiency of heterozygotes, thus the overall inbreeding coefficients in these two species were 2.9 and 14.1 percent respectively. In both the Warkworth population of totara, and the Lake Matheson population of totara var. waihoensis, the fixation index for SDH-2 showed a significant (p=0.05) deficiency of heterozygotes at that locus. As just the one locus had a significant 'F' value, SDH-2 may not be subject to Mendelian inheritance in these species, and its expression

may be influenced by the environment or by some other gene. Thus it appears that there is substantially more inbreeding in the Christchurch kahikatea population than in any of the totara species populations.

DISCUSSION

As previously mentioned, the results from this work should be regarded as preliminary due to the limited sampling of all species involved. Poor seed production and germination led to most populations being smaller than desirable; however it has been calculated that estimates of within-population heterozygosity based on ten-seed samples of eight to twelve individuals are usually within one percent of the actual value, and even two individuals give a good estimate of heterozygosity (Gorman and Renzie, 1979). Although a ten-seed sample will identify a heterozygous locus in an individual with a probability of $p=0.998$, while the eight-seed samples of totara give a probability of $p=0.992$ and the six seed samples of rimu a probability of $p=0.969$, the populations in this study should give an adequate initial overview of the genetic variability in rimu, kahikatea and the totara species.

In all species there was a large amount of inter-locus variability in expected heterozygosities, ranging from 0 to 0.58. This indicates that, as expected, the individual isozymes do not have equivalent contributions to the mean heterozygosity of the populations. For this reason, many isozyme loci should be used to obtain a reliable estimate of variability.

Since the number of maternal parents in all seed collections was limited and the origins of the paternal contributions were unknown, the assumption that the allelic frequencies observed in the megagametophytes and embryos reflect those in the entire populations could be invalid. For this reason, and because expected heterozygosity is calculated on the assumption that the population is in Hardy-Weinberg equilibrium, the figures for expected

heterozygosities of the various loci should be interpreted fairly cautiously. This is especially true for kahikatea, where observed heterozygosity in the Christchurch sample population was significantly different from that predicted if the entire population is in Hardy-Weinberg equilibrium. Conditions for Hardy-Weinberg equilibrium include a population of infinite size with randomly mating, non-overlapping generations with no mutation, selection or migration; none of which can be ensured for the populations in this study.

Variation Within and Between Populations

In general, conifers are one of the most genetically variable groups of plant species (Mitton, 1983). They are often long-lived, late successional, out-crossing, wind pollinated species, and have large geographic ranges and high fecundities. These traits are common in plants with high levels of variability (Mitton, 1983; Hamrick et al., 1979).

Most of this variation, however, is found within individual populations of the coniferous species. Low levels of allozymic differentiation of populations are partly due to the mating system of conifers (Hamrick, 1983), as wind pollinated, out-crossed species have enhanced potential for gene flow. Levin (1986) reports that wind pollinated plant species average six percent of their variation between populations; while self-pollinating species average 44 percent. In general then, the majority of the genetic variation in conifer species resides in the species as a whole (ie. within populations) while a much smaller percentage of variation is due to population differentiation.

In the sample populations of rimu, kahikatea and the totara species, between 3.0 and 8.5 percent of the existing variation was due to allozyme differences among populations. These figures correspond to the range in other species. For instance, the percentage of the total variability among populations is between two and four percent in lodgepole pine and jack pine (Dancik and Yeh, 1983; Yeh and Layton, 1979), three percent in pitch pine (Guries and Ledig, 1982), four

percent in ponderosa pine (Linhart et al., 1981) and incense cedar, and five percent in radiata pine (Ledig, 1987). Douglas-fir is an intermediate species with a Gst of eight percent (Yeh and O'Malley, 1980). Species with a larger proportion of their variation among populations are big tree (10 percent) (Fins and Libby, 1982), Monterey cypress (12 percent), Jeffrey Pine and bishop pine (14 percent) (Ledig, 1987), and western white pine (14.8 percent) (Steinhoff et al., 1983). In many cases, species with more variation among populations are those with reduced ranges and geographically distinct populations. These attributes result in reduced gene flow among populations and an accumulation of genetic differences.

Variation that is present between populations in the heterozygosities of the various loci can be produced by differences in mutation rate or by natural selection (Yeh and O'Malley, 1980). Some support for the theory that selection pressures are influencing the levels of heterozygosity in rimu, kahikatea and snow totara was given by the correlations of heterozygosities with climatic and geographic variables. With migration and hybridization of certain genotypes, the same correlations could be produced for neutral alleles, but for randomly chosen loci, there should be no correlations. The loci chosen in this study were selected in a random fashion; thus the fact that some correlation of heterozygosity with climate was found, and that in some cases the heterozygosity in one enzyme was correlated with the same environmental variable in different species, suggests that these loci are selectively important.

Divergence of Populations

Genetic distance figures express the accumulated number of gene differences per locus between populations if the rate of gene substitution per locus is the same for all loci (Nei, 1972). If the rate is not the same, the number of gene substitutions per locus is underestimated. This measure is applicable to any organism, regardless of ploidy or mating scheme.

Isolation by distance commonly leads to population differentiation, in which case geographic and genetic distance are often positively correlated (Yeh and O'Malley, 1980). This was not true for the rimu, kahikatea or snow totara populations. Neither were the genetic differences between the individual populations within species significantly different, perhaps not surprising in species with such limited genetic variability. Although the genetic distances were not significant, it is interesting to note that the dendrogram for rimu populations (Figure 15) roughly split the populations into northern and southern groupings. This could reflect the split of northern and southern forests by heavy glaciation in the central South Island between 75,000 and 15,000 years B.P.; and it would be interesting to do a more complete sampling of rimu and kahikatea populations to test this observation.

In the dendrogram of totara species (Figure 16), the apparently random divisions of the populations within species indicated the insignificant genetic differentiation between populations. The clustering of all populations within a species indicated the greater genetic differences between species groups. The fact that the two populations of Hall's totara were so widely separated may be a result of the small population sizes. It might also be that the samples contained hybrid material, causing them to be more genetically similar to other totara species than to each other.

Variation Between Species

From this analysis, rimu and kahikatea seem to be significantly less genetically variable than the totara species. Although there were no significant differences between the totara species, on average snow totara and totara were the least variable, while Hall's totara and totara var. waihoensis were the most variable. This finding for Hall's totara is consistent with the flavonoid analysis of Markham et al. (1985).

On global terms, Podocarpus appears to be a more variable genus than either Dacrydium or Dacrycarpus. There are 110 species of Podocarpus, while there are only 22 species of Dacrydium and 9 species of Dacrycarpus (Sporne, 1965; deLaubenfels, 1969). This greater variability may be due in part to a larger number of chromosomes in the genus Podocarpus.

The Podocarpaceae have a surprisingly large variation in chromosome numbers compared to other families of gymnosperms (Hair and Beuzenberg, 1958). Most species in the genus Podocarpus have a diploid complement of 34 chromosomes and snow totara has 38 chromosomes. Both rimu and kahikatea have a total of only 20 chromosomes (Hair and Beuzenberg, 1958). Hamrick et al. (1979) found a strong, positive relationship between chromosome number and variability detected by electrophoresis in a survey of 110 plant species. Species with high chromosome numbers would be expected to have increased recombination, thus their progeny would be more genetically variable (Hamrick et al., 1979).

An alternative factor which might contribute to the reduced variability in kahikatea is that it appeared to be significantly inbred. Although the entire stand of kahikatea in Christchurch consists of only 480 individuals (D. Norton, pers. comm.), the ancestors of these trees were part of a much larger forest. In pre-Polynesian times, forest extended over the Canterbury plains from the coast to the Main Divide mountains (Molloy, 1969). While kahikatea occurred only on the deeper, well-drained soils of the plain and inland valleys, it was among the main species in the coastal swamp and semi-swamp areas (Molloy, 1969). These swamp forests stretched almost continuously from the Hurunui River to south of Lake Ellesmere, and then discontinuously to Timaru (Johnston, 1961). The maximum development of this forest occurred between 5,000 and 3,000 years ago (Molloy, 1969).

Large fires in Canterbury, beginning 900 to 1,000 years ago, destroyed much of the podocarp forests and the regeneration following these fires was often beech (Molloy, 1969). When Europeans arrived, the podocarp forest was

restricted to Banks Peninsula and a few small patches on the plains (Johnston, 1961). In 1850, Dean's Bush was only 21.5 ha in size, and in 1914, 6.1 ha of this forest was reserved (Wall, 1923). Thus while the genetic study of kahikatea remaining in Dean's Bush is of a limited nature, it is unlikely that the high level of inbreeding is entirely a result of the present small population. The trees present today would have regenerated when the stand was almost four times its present size, and previous generations would have been part of a much larger forest. There is a negative association between the amount of inbreeding and genetic variation (Hamrick et al., 1979); thus if the apparent inbreeding found in the Christchurch sample population applies to all kahikatea populations, it could explain why this species is so much less variable than the predominantly out-crossing totara species.

Other Analyses of Variation in New Zealand Podocarps

The finding of low levels of variation within rimu is supported by work done with flavonoid analysis (B.P.J. Molloy, pers. comm.). Morphological variation in rimu populations has been observed, but quantification is lacking (Norton et al., 1988). Variation in foliage, branch angle, presence of the receptacle, and colour of the seed have been reported (McEwen, 1983; Beveridge, 1983), however no differences in the pattern of seasonal growth have been noted.

Kahikatea possesses a highly distinctive pattern of flavonoids which distinguishes it from other members of the Podocarpaceae. This pattern was found to be constant in several widely separated populations (Markham et al., 1985). No documentation of morphological variation in kahikatea could be found, and Markham et al. (1985) refer to it as a 'uniform' taxon.

Extensive flavonoid analysis of all species of Podocarpus and many of their hybrids has been carried out (Markham et al., 1985; Webby et al., 1987). According to these studies, the species of Podocarpus are not as well

distinguished chemically from one another as are those in other genera, but the species can be separated by different marker compounds. Variation in flavonoid patterns between samples in each species was generally found to be small, with Hall's totara showing the greatest variability (Markham et al., 1985). Morphologically, the species of totara are highly variable, yet they appear to hybridize readily (Poole and Adams, 1980).

Significant variation has been found within and between rimu populations in levels of foliage diterpenes and sesquiterpenes (Perry and Weavers, 1985; Berry et al., 1985). It was concluded that the production of these compounds was under genetic control, and that this was related to geographic origin. Geographically separated chemical races of North American conifers have been explained in terms of the distribution of the species in the past; and it was suggested that the discovery of distinct populations of rimu could give evidence of the course of reforestation after the last glaciations (Perry and Weavers, 1985).

While such chemical analysis of conifer foliage may be useful for separating populations, it does not give a very good estimate of the overall levels of variation within the species. From one to five diterpenes or sesquiterpenes may be produced by one enzyme system (Berry et al., 1985), thus variability may be emphasized by looking at these chemicals alone, and no account is taken of the many other enzyme systems. Therefore, for an overall estimate of genetic variability in a species and its populations, isozyme analysis of a large number of randomly selected loci gives a better result.

Variation in Relation to Other Conifer Species

While other conifers average 27 percent heterozygous loci per individual (S.E.=4.1) (Mitton, 1983; Hamrick et al., 1979), all species in this study averaged less than half this value which is a significant difference ($p=0.05$). Using the 95 percent criterion of polymorphism, the average percentages of polymorphic loci for the podocarp species studied were

also significantly less (p=0.05) than the average 67.01 percent (S.E.=7.99) of polymorphic loci in other gymnosperms (Hamrick et al., 1979). Although these figures are not strictly comparable as the enzyme systems and number of loci sampled were different, they do indicate that in comparison with other coniferous species, the rimu and kahikatea populations analyzed are at the low end of the range of variation, and even totara is significantly less variable. Other conifers with very low levels of variation are Torrey pine (Ledig and Conkle, 1983), western red cedar (Copes, 1981) and red pine (Fowler and Morris, 1977).

The low percentages of polymorphic loci in rimu and kahikatea were a result of most loci having only one allele. The average number of alleles per locus for gymnosperm species in general is 2.12 (S.E.=0.2) (Hamrick et al., 1979) which is significantly greater (p=0.05) than the figures obtained for all species in this study.

A Discussion of the Podocarp's Reduced Variation

Despite the significant differences in variability between the New Zealand species, all were significantly less variable than 'average' conifers elsewhere in the world. This reduced variability could be due to New Zealand's exceptionally stable climate, as the surrounding ocean buffers the islands from climatic fluctuations. While the continental areas of North America, Europe and Asia underwent extreme changes of climate during the Ice Ages, annual temperatures in New Zealand were depressed by 4.5°C (McGlone, 1985). This was enough to cause deforestation over much of the South Island, however forests survived in areas of the North Island, and probably on newly formed coastal areas on the South Island which were exposed as the sea level dropped (McGlone, 1985). Thus New Zealand species may have always been growing in a relatively equable, oceanic climate which could have resulted in stabilizing selection eliminating extreme genotypes and reducing genetic variability. This process would have continued even more effectively after the glaciations.

Another reason for the reduced variability of New Zealand's coniferous species could be genetic 'bottleneck' effects. Low variability within populations of other conifers such as Torrey pine and red pine is attributed to reduced population sizes during the species' past, resulting in 'evolution bottlenecks' (Ledig and Conkle, 1983). The rate of genetic drift increases with decreasing population size (Kimura and Ohta, 1971) and this leads to a more rapid reduction of genetic variation within small populations. Even if the small populations later increase in size, it will still take many generations for enough mutations to occur to recreate the original variability (Ledig and Conkle, 1983). Thus if the ranges of rimu, kahikatea and totara species had been reduced at some time in the past, with the species divided into a few isolated pockets of individuals, reduction of variation within these small populations could have occurred quite rapidly. If the species then spread once again and these small populations were reunited, the mating system of the species would lead to gene flow between populations and an even spreading of the remaining genetic variation over all populations. If the contraction and expansion of the populations occurred several times in alternating cycles, it could lead to the present situation of species with low overall variability attributable mainly to variation within populations.

The Relation of Genetic Variation to Geologic History

The cyclical contraction and expansion of podocarp populations has been occurring in New Zealand in the past two million years. In this period there have been at least 20 glacial-interglacial alternations; giving rise to a pattern of cool, largely deforested periods alternating with warm periods when forest cover was nearly complete (McGlone, 1985). The glacial cycles have been further broken into stadial-interstadial periods. McGlone (1985) recounts how the last Ice Age (c. 100,000 to 10,000 years B.P.) was a complex event comprised of at least three major interstadials during which forest occupied most of New Zealand. These

interstadials were separated by severe intervals during which forest was extremely restricted throughout the country. The most recent of these stadial periods was the last glaciation which was at a maximum 25,000 to 15,000 years ago.

These alternating periods of glaciation and amelioration could have been the force which reduced genetic variation in podocarp populations. During the last 75,000 years the populations have been restricted at least three times, each time possibly resulting in a further rapid reduction in variation. Between cold periods, the populations would have expanded again which would have led to gene flow and a distribution of the remaining variation among populations. The potential for gene flow through a series of contiguous subpopulations of podocarp species exists with the synchronous flowering associated with mast seeding, the long distance flight of coniferous pollen, and the possibility of avian seed dispersal. Thus, mechanisms for reducing variability in podocarp populations may have been present and active for at least two million years.

Although variability may have been reduced during glacial periods, at some point the alpine and subalpine podocarp species must have evolved in response to selection pressures for cold tolerance. If the genus Podocarpus was initially more variable than either Dacrydium or Dacrycarpus due to greater numbers of chromosomes, more outcrossing, more frequent and abundant seed production, or other factors, it would have enabled that genus to change more rapidly in response to environmental change. This appears to be true as there are Podocarpus species growing at every altitude from sea level to the subalpine.

The divergence of rimu and pygmy pine also led to one species becoming adapted to alpine habitats while the other remained in lowland forests; however this divergence does not appear to be as recent as the divergence of Podocarpus species (Figure 17). Pygmy pine is chemically and taxonomically distinct from rimu (Quinn, 1982), which indicates that these species diverged much longer ago than the Podocarpus species. Further evidence for the recent

evolution of Podocarpus species is given by the fact that they hybridize readily (Poole and Adams, 1980). This ability to hybridize would have helped maintain variability in the genus as a whole, and although populations of all species may have been restricted in cold periods, any variability lost from the genus Podocarpus may have been more quickly replaced by hybridization. This is perhaps why the totara species are now more variable than either rimu or kahikatea.

SUMMARY

The isozyme analysis of the three podocarp species rimu, kahikatea and totara provided some new information on the genetic variability within and between the species. All of the New Zealand conifer species studied were significantly less variable than their average northern hemisphere counterparts. This may be due to New Zealand's temperate climate or to evolutionary bottleneck effects caused by expanding and contracting populations during the Ice Ages.

Reduced variability may affect the speed and degree to which these species can respond to selection pressures. The correlations of heterozygosities of some loci with climatic variables indicate that variability has been influenced by the species' response to selection pressures, but no unusual degree of differentiation between populations has occurred.

The totara species were significantly more variable than either rimu or kahikatea. This may be due to differences in chromosome numbers, the degree of inbreeding in the species or the amount of hybridization. Again, it might be expected that the greater variability would enable the totara species to react more quickly to changes in selection pressures.

CHAPTER VII

FROST EXPERIMENT

INTRODUCTION

The high temperature and totara provenance experiments indicated that in the increasingly cool climates of the past two million years (McGlone, 1985), selection pressures have no longer been acting on growth rate. Isozyme analysis, however, indicated that the small amount of genetic variation among populations of rimu, kahikatea and snow totara may well be associated with some type of current climatic selection pressures.

In other species, variation in the tolerance of low temperatures is usually even more closely tied to the climate of seed origin than is optimum growing temperature (Ewusie, 1980). While some tropical tree species may be killed by temperatures above $0^{o}C$, most trees can tolerate a few degrees of frost, and boreal conifers can withstand temperatures lower than $-40^{o}C$ (Bannister, 1976).

In the cool climatic regimes of the past two million years, if selection pressures have not been acting upon the growth rates of the New Zealand podocarps, they are more likely to have favoured cold tolerance. In an effort to confirm this, an experiment was designed to test the frost tolerance of various provenances of rimu, kahikatea and totara; and to explore the possibility of allele frequency-dependent selection for this trait.

MATERIALS AND METHODS

Seedling Origins

Thirty-six seedlings of each of several provenances of rimu, kahikatea and totara were raised in the glasshouse on

capillary benches at a minimum temperature of 22°C. Warm temperatures were required to promote the growth of the rimu and kahikatea seedlings so that they would reach a suitable size to undergo freezing.

The seedlings were potted in plastic planter bags, the totara in PB 1 1/2 bags and the other two species in PB 1 bags. The bags were filled with Odering's Nine Month Potting Mix.

The provenances of the various species were as follows (see Appendix B for details of origin):

Rimu: Kaikohe, Central North Island, Hokitika Jackson Bay, Waitutu

Kahikatea: Whirinaki, Charleston, Christchurch

Totara: Kauaeranga, Pureora, Whangamomona, New Plymouth, Taihape, Hunterville, Pohangina, Otaki

Development of Frost Hardiness

To induce the development of frost hardiness in conjunction with the natural seasonal cycle, at the beginning of May the totara seedlings were transferred to another glasshouse heated to a minimum of 12°C. The seedlings were held there for two weeks, and subsequently, the heating was reduced to a minimum of 9°C for two weeks, and then to 4°C for one month. At this time, the seedlings were removed to an unheated shade house with a minimum temperature of -3°C and a maximum temperature of 11°C. The totara seedlings remained there for eight weeks.

The rimu and kahikatea were smaller than the totara seedlings, and so were maintained in the glasshouse at a minimum temperature of 22°C for an extra eleven weeks to increase their size. After the middle of May, daylight hours were extended using 400 W high-pressure sodium lamps so that plants were subjected to 16 hours of daylight.

At the end of July, the rimu and kahikatea seedlings were transferred to another glasshouse to undergo the same

hardening process as the totara. For these species, however, time at the 9°C minimum temperature was reduced to one week. Five days after the 4°C minimum temperature was reached, the seedlings were removed.

Seedlings of all species were transported by road at the end of August to the DSIR Climate Laboratory, Plant Physiology Division, Palmerston North. Here the totara were repotted in a mixture of: 50% Opiki loam, 37% fine gravel and 13% coarse pumice with 0.16% (w/v) Nitrophoska and 0.4% dolomite lime; and subjected to the frost regimes.

Rimu and kahikatea seedlings were transferred to a C.E. room for 27 days to increase their frost hardiness. The day temperature of the C.E. room was $11.0^{\circ} \pm 0.5^{\circ}C$, and the relative humidity, 54.3 percent. This was followed by night conditions of $3.0^{\circ} \pm 0.5^{\circ}C$ and 60.4 percent. The diurnal change in temperature and relative humidity was programmed to occur at a constant rate over 11.5 hours with a half hour steady state condition at the two extremes. Lighting was as described in Greer and Warrington (1982). The photoperiod was ten hours and was centred around the time of the 11°C daily maxima. After the 27 days, the rimu and kahikatea were removed, repotted in the same mixture as the totara, and subjected to the frost regimes.

Experimental Design and Frost Conditions

Three frost temperatures were chosen on the basis of the results of test frosts of -8°C run with a few seedlings of each totara provenance. The test frost for rimu and kahikatea seedlings was run at -9°C.

For totara, the frost temperatures were set for -3°, -6° and -9°C, run on a 6-6-4 program; that is, six hours to cool from 12°C, six hours at the minimum temperature, and four hours to warm again to 12°C. Each frost temperature was replicated twice. Actual mean frost temperatures for all the replications were: -2.7°, -2.8°, -5.8°, -5.8°, -9.0° and

-9.0°C. Relative humidity was kept at 100 percent during the frost, and 60 percent when temperatures were above 0°C. The majority of the frost was carried out in darkness, but the lights were turned on one hour before warming was completed (Plate 13) (for a description of the frost rooms see Robotham et al., 1978).

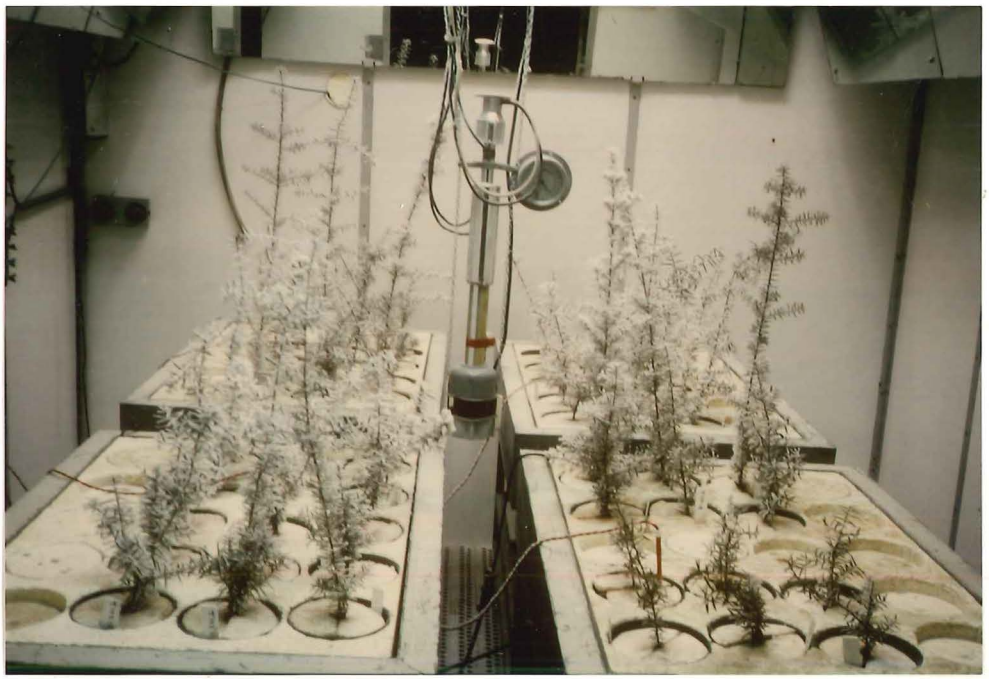

Plate 13: Totara seedlings during -9°C frost

The rimu and kahikatea were tested under the same frost conditions as were the totara seedlings. Actual mean frost temperatures for the replications were: -2.8°, -2.8°, -5.9°, -5.9°, -9.0° and -9.0°C.

The 36 seedlings of each provenance were grouped by descending height into units of three trees. These three tree units were kept together and treated as one experimental unit. Two units were chosen randomly from each provenance for each frost, and the units of the various provenances were arranged randomly within the frost rooms.

Measurement of Damage

For frost damage evaluation, the seedlings were placed in an outdoor shelter, the totara for six weeks and the rimu and kahikatea for three weeks, when foliage damage was assessed visually. Damage was categorized on a scale as follows (Menzies, 1977): 0 = no damage
1 = some needles slightly damaged
2 = 10 to 30% of needles killed
3 = 50% of needles killed
4 = 90% of needles killed
5 = seedling dead

Analysis

The scores for the seedlings in each experimental unit were averaged, and a split-plot analysis of variance carried out with these scores and also with the arcsin transformation of the mean percent of foliar damage of each unit. The analysis model for these variables for the totara seedlings was as follows (see Appendix V for sums of squares):

	SOURCE OF VARIATION	df
Whole plot	Temperature Treatment	2
	Replicate	1
	Error A	2
Split-plot	Provenance	7
	Provenance X Replicate	7
	Provenance X Treatment	16
	Treatment X Replicate X Provenance	14
	Error B	48
	Total	97

The 'Error B' term was comprised of the following elements:

SOURCE OF VARIATION	df
Between Units	1
Treatment X Units	2
Replicate X Units	1
Provenance X Units	7
Treatment X Replicate X Units	2
Treatment X Provenance X Units	14
Replicate X Provenance X Units	7
Treatment X Replicate X Provenance X Units	14

The variation explained by these interactions was insignificant, thus these terms were pooled to increase precision. The results of the frosts with rimu and kahikatea seedlings were analyzed the same way, but then a 'Species' term and appropriate interactions were also included in the model (Appendix V). The results from the Central North Island rimu provenance were not included as there were too few seedlings. The means for each species and frost temperature were compared using Scheffe's test with a confidence level of 0.95.

The percentage of trees severely damaged at each frost temperature was calculated by allocating a value of '0' to those units with damage scores of 2 or less, and a value of '100' to those units with damage scores of 3 or more. These values were then averaged for the units of one species at one frost temperature.

It has been determined for radiata pine (Menzies et al., 1981) and field grown Acacia seedlings (Pollock et al., 1986) that a frost temperature producing a damage rating of 2 is the maximum level of frost from which a seedling can recover; thus this has been specified as the seedling frost hardiness temperature. This measure was calculated for the rimu, kahikatea and totara provenances by interpolating between the mean damage ratings at the various temperatures. The seedling frost hardiness temperature results were then correlated with the elevations and latitudes of seed origin.

Isozyme Analysis

Isozyme analysis, using the methods described in Chapter VI, was carried out with the new, spring foliage of seven trees from the same totara provenances used in the frost experiment. Allele frequencies of the four, scorable, variable loci were calculated for each provenance, and a correlation analysis was run between the allele frequencies and frost hardiness of the totara provenances.

RESULTS

There were no differences in the results of the analyses of variance using the actual damage scores or the arcsin transformation of percent damage. The rest of the discussion, therefore, will deal with the analyses of damage scores.

Frost Damage

For all species, frost damage was significantly affected by temperature ($p=0.0001$) with increased damage at lower temperatures; and by provenance ($p=0.0001$). There was also a significant ($p=0.0001$) temperature X provenance interaction for all species, as the ordering of provenances with respect to damage changed at different temperatures. This did not affect the final interpretation, however, as rimu and kahikatea seedlings virtually all survived at $-3^{o}C$ or all died at $-9^{o}C$; thus the only useful temperature for comparison of these provenances was $-6^{o}C$. For the totara seedlings, the extent of damage was interpretable at both -6^{o} and $-9^{o}C$ frost levels; but while the ordering of some provenances changed at these temperatures, the differences in damage between these variable provenances were not significant ($p=0.05$). Table 36 presents the frost damage scores and percentage of severely damaged trees for the three species at the three frost temperatures.

For the frosts of the totara provenances, the difference between replicates was significant ($p=0.001$); however this source of variation was not significant for the frosts of the rimu and kahikatea seedlings.

As the totara seedlings had been treated quite differently from the seedlings of the other two species, their damage scores were analyzed separately. It was obvious however, that the totara provenances in this trial were much hardier than the rimu or kahikatea provenances as many totara

survived the -9°C frost with comparatively little damage. There was a significant (p=0.0001) difference in frost tolerance between the rimu and kahikatea seedlings which had been treated in the same manner. The kahikatea provenances appeared to tolerate the -6°C frost somewhat better than the rimu provenances.

Table 36: Mean frost damage scores and percent of severely damaged trees for each species and frost temperature [1]

	-2.8°C		-5.8°C		-9.0°C	
	Damage Score	%Severely Damaged	Damage Score	%Severely Damaged	Damage Score	%Severely Damaged
RIMU						
Kaikohe	0.16	0	4.58	100	4.79	100
Waitutu	0.25	0	4.42	100	4.75	100
Hokitika	0.16	0	3.75	100	4.92	100
Jackson B.	0.25	0	3.75	100	4.66	100
KAHIKATEA						
Whirinaki	0.16	0	2.75	58	4.83	100
Christch.	0.16	0	2.25	33	4.75	100
Charleston	0.25	0	2.75	58	4.92	100
TOTARA						
Otaki	0.42	0	2.58	50	4.75	100
Kauaeranga	0.00	0	1.75	17	4.25	83
New Plym.	0.16	0	1.62	8	3.92	100
Pohangina	0.25	0	1.42	8	3.17	69
Huntervil.	0.25	0	0.92	0	3.16	67
Pureora	0.00	0	0.75	8	3.08	75
Whangamom.	0.08	0	0.75	0	2.00	17
Taihape	0.00	0	0.58	0	1.75	17

[1] Means enclosed by the same bar are not significantly different (p=0.05).

Comparing the frost tolerance of provenances within species, the totara seedlings from Kauaeranga, New Plymouth and Otaki were the least frost tolerant. Seedlings from Whangamomona and Taihape were the most frost tolerant, and

those from Pureora, Hunterville and Pohangina were of intermediate tolerance (Plate 14).

The differences in frost tolerance between provenances of rimu and kahikatea were not as large as for totara. Rimu seedlings from Hokitika and Jackson Bay were slightly more tolerant of frost than those from Kaikohe and Waitutu, while the kahikatea seedlings from Christchurch were somewhat more frost tolerant than those from Whirinaki or Charleston (Plate 15).

Plate 14: Average frost damage of totara provenances at -6°C (see Appendix B for provenance names and numbers)

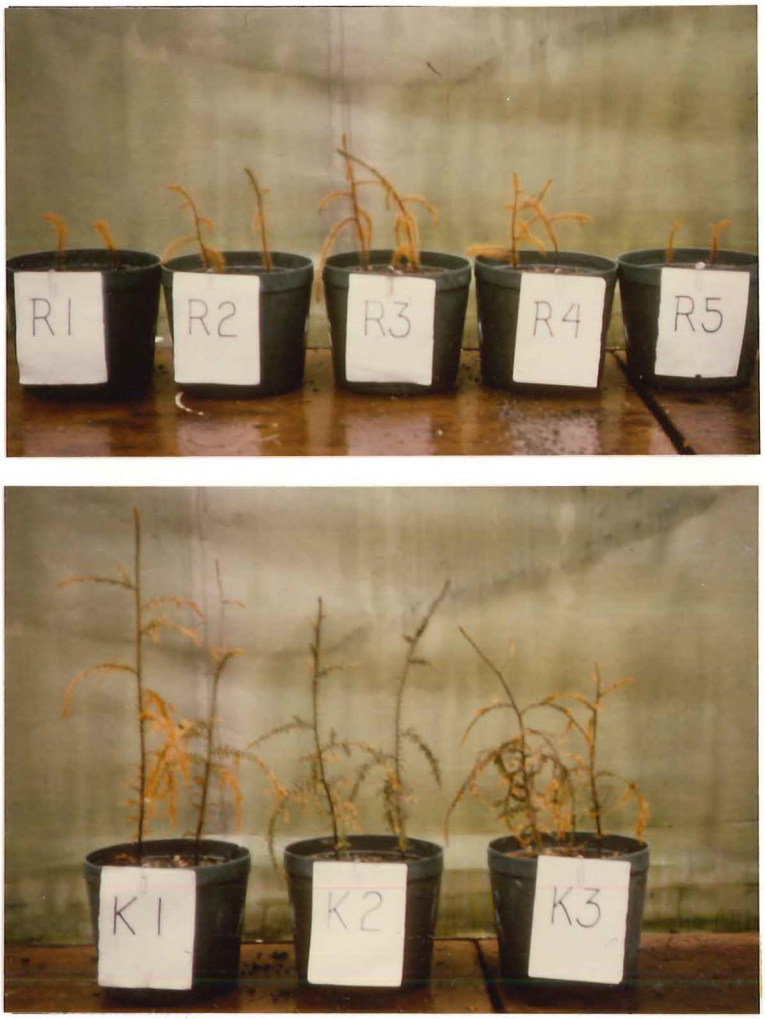

Plate 15: Average frost damage of rimu and kahikatea provenances at -6°C (R1=Kaikohe, R2=Hokitika, R3=Jackson Bay, R4=Waitutu R5=Central North Is., K1=Whirinaki, K2=Christchurch, K3=Charleston)

Frost Hardiness

Table 37 presents the seedling frost hardiness temperatures for the various provenances of the three species. These frost hardiness temperatures demonstrate the comparative hardiness of the three species and their provenances. For the provenances tested, it can be seen that the range of hardiness is much greater for the totara than the other two species.

When the frost hardiness temperatures were correlated with the elevations and latitudes of origin, no significant correlations were found for rimu and kahikatea. Totara demonstrated a positive correlation of frost hardiness with elevation of seed origin (p=0.04, df=7) (see Appendix B for elevations of seed origin).

Table 37: Provenance seedling frost hardiness temperatures

Rimu		Kahikatea		Totara	
Provenance	Temp °C	Provenance	Temp °C	Provenance	Temp °C
Kaikohe	-4.1	Charleston	-5.0	Otaki	-5.0
Waitutu	-4.1	Whirinaki	-5.0	Kauaeranga	-6.1
Jackson Bay	-4.3	Christch.	-5.5	New Plymouth	-6.3
Hokitika	-4.4			Pohangina	-6.9
				Hunterville	-7.3
				Pureora	-7.5
				Whangamomona	-9.0
				Taihape	-9.7

Isozyme Analysis

Of the eight gene loci found to be variable in the seed of two populations of totara in Chapter VI, only three were scorable when foliage was used as source material. These loci were DIA-1, IDH-1 and G6P-1. A new enzyme locus, MR-1, was found to be present in the totara foliage (see Appendix U for stain recipe). Unfortunately AAT, which was correlated with days of frost in the isozyme analysis of both rimu and kahikatea, could not be scored in totara foliage.

Of the scorable loci, the allele frequencies for IDH-1 were significantly (p=0.004) correlated with the frost hardiness of the totara provenances, with the slow allele being more common in frost hardy provenances. Table 38 presents the allele frequencies for IDH-1 in each provenance.

Table 38: Allele frequencies for IDH-1 in eight totara provenances

Provenance	Alleles	
	Fast	Slow
Otaki	1.0	0
Kauaeranga	1.0	0
New Plymouth	0.50	0.50
Pohangina	0.94	0.06
Hunterville	0.61	0.39
Pureora	0.56	0.44
Whangamomona	0.17	0.83
Taihape	0.12	0.88

DISCUSSION

Development of Frost Hardiness

In many plants, tolerance of frost varies significantly over the year. At bud burst, plants have virtually no tolerance of sub-zero temperatures (Cannell and Sheppard, 1982), and over the summer growing period, temperatures more than a few degrees below 0°C cause serious damage (Kozlowski, 1971). In the autumn, plants begin to increase in frost tolerance to a mid-winter maximum. This increase in frost tolerance is induced by reduced photoperiods and low temperatures (Weiser, 1970).

It has been thought that the development of frost tolerance occurs in three stages, with the first stage being initiated by short days and warm temperatures, the second by frost (Weiser, 1970, Kozlowski, 1971), and the third by protracted freezing or by temperatures lower than -10°C (Bannister, 1976). Currently, however, some authorities believe that the hardening process occurs gradually, rather than in stages, with photoperiod and low temperatures exerting influence together throughout the process (D. Greer, pers. comm.).

In the spring, dehardening of plant tissue is induced by increasing photoperiods and temperatures (Greer and Stanley, 1985). Once this dehardening process has begun, plants will not re-harden fully regardless of the regimes of photoperiod and temperature provided (Weiser, 1970).

In this experiment, the rimu and kahikatea may have been more heavily damaged than the totara because of the difference in hardening regimes. Sakai et al. (1981) found that the mid-winter freezing resistance of the leaves of kahikatea and totara was -7°C and rimu was -8°C. While the results from the totara in this experiment were in agreement with the findings of Sakai et al. (1981), the results from the rimu and kahikatea were not.

The totara had been under natural photoperiods over the whole winter and had also been subjected to a series of -3°C frosts while in the shade house; thus these trees would probably have reached a frost hardy state. The rimu and kahikatea, on the other hand, had been induced to increase their size under prolonged photoperiods and warm temperatures for most of the winter. The seedlings of these two species were not subject to natural photoperiods until the end of July when day length would have been increasing.

These seedlings also were never subject to sub-zero temperatures. For a month before the frost, they were held in a regime in a C.E. room that was intended to promote hardening, and their foliage did become red in colour indicating winter dormancy (McEwen, 1983). It does not appear, however, that these species achieved a very frost hardy condition. Cannell and Sheppard (1982) report that Sitka spruce seedlings which are kept in long day conditions do not harden as rapidly as trees in natural photoperiods. Also, Weiser (1970) states that the second stage of acclimation will not develop without sub-zero temperatures; thus the rimu and kahikatea seedlings would not have been as frost hardy as the totara.

Even if the rimu and kahikatea had developed some degree of frost hardiness, this might not have been maintained during the four weeks in the C.E. room. Radiata pine in New Zealand begins to deharden naturally in July, and once this process starts, it cannot be stopped unless temperatures below 6°C are continuously imposed (Greer, 1983; Greer and Stanley, 1985). If lengthening photoperiod or warm temperatures had triggered dehardening in the rimu and kahikatea seedlings, the regime in the C.E. room might not have been severe enough to stop or reverse the process. Thus, the rimu and kahikatea seedlings could not have hardened as much as the totara seedlings, and they may have been dehardening for an extra month. This may explain why these results are different from those of Sakai et al. (1981).

Correlations of Frost Hardiness and Seedling Origins

While there is significant variation in the frost tolerance of rimu and kahikatea provenances, this does not appear to be linked to geographic variables. The variation in the frost tolerance of totara provenances does, however, seem to be related to the elevation of seed origin, with provenances from higher altitudes being more frost tolerant.

The fact that the rimu and kahikatea were not completely hardy could explain the lack of correlation between frost tolerance and provenance origin. Rook et al. (1980) found that Eucalyptus regnans did not show significant differences in tolerance between provenances when frosted in spring, whereas significant differences were present in autumn and winter. Rimu and kahikatea did show significant differences in frost tolerance between provenances; and although there was no correlation of hardiness with elevation or latitude, a weak trend of increased tolerance with harshness of climate was observed. This trend might have

been more significant if the seedlings were completely hardened.

The isozyme analysis of Chapter VI showed totara to be more genetically variable than either rimu or kahikatea. Presumably this greater variability would enable totara to respond more quickly to selection pressures than the other two species. Perhaps this may contribute to the explanation of totara's close correlation of climate and frost hardiness.

The trend shown by the totara seedlings of a strong, positive correlation of frost hardiness and elevation has been demonstrated in many other species such as big tree (Guinon et al., 1982), western hemlock (Kuser and Ching, 1980), Eucalyptus regnans (Rook et al., 1980) and Acacia species (Pollock et al., 1986). Other species demonstrate a correlation of frost tolerance with latitude of seed origin, and lodgepole pine is an example of this type of adaptation (Jonsson et al., 1986).

As well as the inter-provenance variation occurring in mid-winter hardiness of species, major differences occur in the rate of autumn hardening and spring dehardening (Cannell and Sheppard, 1982). Often provenances from southerly latitudes or low elevations continue their growth later in the autumn and begin their growth earlier in the spring than their counterparts from harsher climates. This would be a subject for further investigation with totara seedlings of various provenances.

Variation Between Replications

The significant variation in frost damage between the two replicates of the -9°C and -6°C totara frosts is difficult to explain. Although the temperature sensors registered only a 0.1°C difference in temperature between frosts, variation within the C.E. rooms may be as great as 0.5°C (D. Greer, pers. comm.); therefore, it is possible that temperature variation caused the difference between

replicates. It is also possible that warm temperatures over the two days between replicate frosts caused enough dehardening to occur in the totara seedlings to make a significant difference.

Kobayashi et al. (1983) found the maximum rate of dehardening of red-osier dogwood to be 2.4°C per day at 20°C while average rates were 1.9°C per day at 24°/18°C for Taxus cuspidata (Zehnder and Lanphaer, 1966), and 0.5°C per day at 21°C for Eucalyptus pauciflora (Harwood, 1981). If totara has the capacity to deharden rapidly as temperatures increase, this could have created the variation between replications.

Isozyme Analysis

Allele frequency-dependent selection in isozyme systems has often been found in animals and insects (Stern and Roche, 1974), and presumably it may also occur in plants. Enzymes are involved in the development of frost hardiness, and changes of enzyme composition, conformation or concentration have a significant effect on cold tolerance (Rosas et al., 1986). In New Zealand, where frosts may occur at almost any time of year, it might be expected that isozymes influencing cold tolerance would be selected with different intensities. The frequency of the slow allele in IDH-1 generally increased with provenance frost tolerance, and whether this is because IDH is directly involved in frost hardiness or is linked to another gene that controls cold tolerance, it would appear that selective forces of the past two million years have modified the genetic structure of totara and created provenances adapted to local frost conditions.

A Comparison With Other Species

In comparison with some New Zealand shrub species (Warrington and Stanley, 1987), totara is relatively frost

tolerant. Comparing totara to other New Zealand tree species however, shows this species to be of average frost tolerance. Sakai et al. (1981) found high altitude shrub podocarps such as Halocarpus bidwilli and snow totara to have freezing resistances of -20° to -23°C, but generally, lower elevation podocarps had freezing resistances of -7° to -10°C.

Variation in the frost tolerance of other New Zealand species seems to be well correlated with the climate of seed origin. Warrington and Stanley (1987) found the differences in frost tolerances of Astelia, Dicksonia, Metrosideros and Phormium to be closely related to the climates in which they grow. Provenance variation in the frost tolerance of several New Zealand tree species was shown to be related to their altitude of origin by Sakai and Wardle (1978); and D. Greer (pers. comm.) found a 7°C difference in maximum winter hardiness of mountain beech seedlings from 460 m and 1100 m elevation.

Compared to plants from the northern hemisphere, New Zealand species have relatively low frost tolerances (Sakai et al., 1979). The oceanic climate of New Zealand lacks large seasonal changes in temperature, but may be subject to sudden reversals of temperature at any time of the year. As frost resistance is induced by low temperatures and lost during warm periods, the frost resistance of New Zealand plants may be expected to be less than that of plants from similar northern latitudes (Bannister, 1984).

SUMMARY

While the relatively mild oceanic climate of New Zealand has not led to the evolution of plants with extreme cold hardiness (Stanley and Warrington, 1987), it appears that the limited frost tolerance that has developed is closely related to the climates where these plants originate. Seedlings of various rimu, kahikatea and totara provenances

seem to have maintained a historical ability for optimal growth at high temperatures, but the cooler climates of the past two million years have obviously produced selection pressures for frost tolerance. The inter-provenance variability in the frost tolerance of totara, and perhaps rimu and kahikatea, is an indication of the specificity and strength of the pressures for adaptation to low temperatures.

CHAPTER VIII

A SUMMARY AND INTEGRATION OF RESULTS

The Podocarpaceae is an ancient family of gymnosperms which arose over 200 million years ago (Miller, 1977). Today's podocarp species are the products of millions of years of evolution which has proceeded under climatic and geologic conditions often very different to those of the present day. Evolutionary pressures at all times would have acted to produce races most 'fit' for the then-current environment; thus today's species inherit a long and varied environmental legacy.

One of the objectives of this thesis was to look at two environmental factors which may have influenced 'evolutionary pressures', namely nutrient availability and growing temperature, in order to explain the present-day distribution of rimu, kahikatea and totara, and the overall geographic dominance of rimu.

The first nutrition experiment indicated that some differentiation among the three species has occurred in their ability to utilize and respond to nutrients. While kahikatea and totara had a rapid response to improved fertility, rimu did not have such a marked reaction. Instead, this species seemed more able to survive and persist on low fertility soils, and sacrificed rapid growth response for tenacity.

With the addition of mycorrhizas in the second nutrition experiment, the growth of all three species was improved, primarily due to the enhanced uptake of phosphorus by the mycorrhizal fungi. Rimu and kahikatea appeared to be more mycotrophic than totara, showing significant improvements in growth with mycorrhizal infection in all nutrient treatments. In totara, the improvement in growth appeared to decline with increasing nutrient supplies.

The germination experiment showed rimu and kahikatea to have different optimum temperatures for germination. The rimu seeds had maximum germination in a $30^{o}/20^{o}C$ temperature regime, while the kahikatea seeds germinated best at $18^{o}C$. For the growth of seedlings however, the three temperature experiments demonstrated that rimu, kahikatea, totara, snow totara, mountain beech and kauri all grow optimally at $27^{o}C$.

While the nutrition experiments may shed some light on the dominance of rimu on sites of low fertility, the results of the temperature experiments do not seem to have much relevance to inter-specific competition in the temperate climates of modern-day New Zealand. It is suggested, therefore, that the ability of rimu, kahikatea and totara to achieve optimal growth at subtropical temperatures evolved during the Miocene, when the forerunners of these species were growing in climates much warmer than today. This characteristic must then have been maintained through subsequent generations of podocarps to the present, and has even been retained in the subalpine podocarp species, snow totara. This implies that the relic trait is neutral in terms of current selection pressures, and that these pressures must have been acting on attributes other than growth rate.

If there has been no change in the adaptation of growth rates in these New Zealand species since the Miocene, there should be no correlation between optimal growing temperature and current climate for provenances of the same species. This hypothesis was tested in the totara provenance experiment; and while some differences in optimum growing temperature were found among the seven provenances, these differences could not be correlated with any geographic or climatic variables. It was therefore concluded that the variation was due to random genetic change and not to selection pressures.

Isozyme analysis showed that, in comparison with North American conifers, the New Zealand conifers studied appear to have low levels of genetic variation. Several factors may be involved in the explanation of the reduced variation in these species.

During the frequent glacial/interglacial cycles of the last two million years, podocarp populations have alternately been contracting and expanding (McGlone, 1985). When population sizes are limited, there can be a rapid loss of variability through genetic drift (Kimura and Ohta, 1971); thus the repeated restriction of populations during glacial phases could have led to the reduced genetic variation in the podocarps.

Once variability had been reduced, the longevity and delayed reproduction of the podocarps would slow the rate of new genetic change in comparison with species that have short generation times (Levin and Wilson, 1978). Another attribute of forest tree podocarps which could reduce their rate of evolution is that they are late successional species, and only form a sparse overstorey in the forest (Wardle, 1964). The few seedlings that survive beneath the canopy may well reflect the chance location of light gaps rather than the intense type of genetic selection which occurs in a pioneering species. Also, the relatively mild oceanic climate of New Zealand would not generate extreme selection pressures to force rapid genetic change.

Isozyme analysis also indicated that the limited variation occurring in rimu, kahikatea and snow totara may be due to varying selection pressures. Since it appears that selection for growth rate has not been significant in the past seven million years, the genetic variation present in these species must be due to other selection pressures.

As world climates cooled from the subtropical conditions of the Miocene to the harsh, glacial episodes of the Pleistocene and Holocene, the survival of all species

must have been greatly threatened by low temperatures. The ability of organisms to develop cold tolerance would have been a survival trait strongly favoured by selection pressures, perhaps to the exclusion of most other traits. The frost experiment indicated that totara, at least, has been under pressure to adapt to cold, and has evolved a tolerance of frost that is related to climate.

The distribution of rimu, kahikatea and totara has not been well explained by the results of these experiments; and perhaps the complex interactions of all factors influencing growth make it impossible to isolate individual causes of species' site preferences. Rimu's dominance as a species may be partially explained by its ability to tolerate low levels of fertility, but the results of the temperature experiments do not distinguish between species.

These experiments do, however, indicate that the forest tree species of New Zealand still maintain some characteristics related to their distant evolutionary past; and that these attributes differ significantly from those of coniferous forest tree species from similar climates in the northern hemisphere, particularly western North America. The major differences between the two floras can be attributed primarily to the comparatively ancient origins of the New Zealand species, and to the differing extent and severity of cooling the two land areas experienced in the Pleistocene.

Apart from substantial differences in the families and genera represented in the two regions, other significant differences occur. The growth and photosynthetic rates of the native forest trees of New Zealand are significantly lower than those of species such as radiata pine from similar climates in North America. Typical mean annual increments for managed native coniferous species in New Zealand are one to five m^3/ha/year (James, 1987), while species introduced from North America average 25 m^3/ha/year (New Zealand Institute of Foresters, 1986). Correspondingly, the light-

saturated rate of net photosynthesis of radiata pine at 20°C is more than four times that of rimu (McEwen, 1983).

The altitude of timberline in New Zealand is also lower than that at comparable latitudes and distance from the coast in North America. The New Zealand timberline species also have less cold resistance, and the trees are angiosperms, rather than the more typical North American gymnosperms.

Other differences between the New Zealand and North American floras demonstrated by work in this thesis are the lack, in New Zealand tree species, of strong, genetically based relationships between growth rates and climates of origin. The differential between optimum growing temperatures and average summer day temperatures was significantly greater for New Zealand forest tree species than for other species from elsewhere in the world. Also, genetic variation appeared lower in New Zealand coniferous forest trees; and all these differences may be linked through a single interpretation based on the contrasting evolutionary history of the flora of New Zealand and North America.

As previously mentioned, the high optimum temperatures for the growth and photosynthesis of New Zealand's forest species may be a relic attribute from the Miocene. As today's average temperatures are below this optimum, this could partially explain why growth rates in New Zealand trees are presently so low, and why, at the intra-specific level they cannot be related closely to climate.

Considerations of climatic changes since the Miocene would suggest that the strongest selection pressures on subtropical tree species could have been to develop cold resistance. That this was achieved in some species is indicated by their survival to the present day, as opposed to the North American subtropical species which disappeared and were replaced by more recently evolved, highly cold tolerant species. Among the surviving New Zealand species, there has

been differing response to the selection pressures for cold resistance. Totara demonstrates a significant intra-specific relationship between the climate of origin and frost resistance, but has not developed a very great tolerance of cold, surviving only to -10°C (Sakai et al., 1981). Snow totara, on the other hand, has responded to the selection pressures more quickly, and while optimum temperatures for growth and photosynthesis have not yet been modified by the cooler climates of the past two million years, this species has developed much greater cold tolerance than its relatives, surviving to -23°C (Sakai et al., 1981).

Although world climates have been cooling for the last seven to ten million years (Frakes, 1979), it is only 1.7 million years since the last 'tropical' taxa disappeared from New Zealand (Mildenhall, 1980). The rapidity of change from subtropical to glacial climates and the development of the subalpine zone in the last few million years (Raven, 1973) must have put New Zealand's species under tremendous pressures to adapt to the new conditions. Subalpine climates have arisen so recently that species have not had enough time to produce highly cold-tolerant races; thus it is not surprising that North American species, which had already experienced more than 10 million years of upland, cool-temperate climates before the Pleistocene, have evolved to withstand much greater cold, and grow to much higher altitudes than New Zealand species (Wardle, 1985).

Reduced genetic variability in the podocarps would make them particularly slow to adapt to rapid changes of climate. They are also long-lived, slow to reproduce and are generally climax species which produce few offspring, so selection pressures have little genetic material on which to act. This might explain why the podocarps have no place as subalpine tree species in New Zealand, whereas conifers usually border the upper elevation treeless zone in other continents (MacMahon and Andersen, 1982). The podocarps that

reach to the subalpine, such as snow totara and pygmy pine, have quite different physiognomies and life histories than rimu, kahikatea and totara; and these differences may enable a faster rate of evolution.

The tree species that dominate New Zealand's timberline are the beeches. Although this family has been present since the late Cretaceous (Fleming, 1975) and has apparently evolved the same 27°C optimum growing temperature as the podocarps, the beeches seem to have responded more quickly than most of the conifers to the selection pressures of the last two million years.

The beeches generally have much shorter life spans than the forest tree podocarps, they reproduce earlier, and they are mostly pioneering species producing large numbers of offspring on which selection pressures may act. They are also associated with ectomycorrhizas, as are northern hemisphere coniferous species, rather than with the endomycorrhizas of the podocarps. This may have bearing on the ability of beech to attain a larger stature and faster growth rates than the subalpine podocarp species (Wardle, 1985). For these reasons, it appears that the beeches have undergone more rapid evolution than the lowland podocarps and have radiated to fill the new ecological niches created by the changing climate and geography of New Zealand.

In Australia, the beeches are also a component of high elevation forests, and Nothofagus gunnii has evolved to be the only cold-deciduous tree in that country. The dominant, high elevation species however, are the eucalypts, which supports the suggestion that the genus with the highest rate of evolution is most likely to fill new ecological niches.

Australian trees have not developed the cold tolerance of North American species either, thus it would appear that the global cooling of the last seven million years has been less severe on South Pacific land masses in general, as compared to North America and Europe. This may have enabled

some primitive species to survive relatively unaltered, while still promoting the development of a degree of cold tolerance in the newer species.

The distinctive attributes of the New Zealand forest tree flora are thus explainable in terms of their extended subtropical history, the subsequent selection pressures placed on them during the Pleistocene and Holocene, and their long isolation from newer, more competitive floras evolving in less equable areas of the globe.

ACKNOWLEDGMENTS

I would like to express my appreciation and thanks to Professor G.B. Sweet for his enthusiasm, encouragement and inspiration throughout this project, and for his help and support in our exploration of New Zealand. Thanks are also due to Mrs. M. Sweet for her hospitality and efforts to make us feel at home in this country, and for her help in the collection of experimental material.

I am most grateful to Mr. D. Bergin of the Forest Research Institute, Rotorua who provided the seed and seedlings for most of the experiments. My appreciation is also extended to Mr. J. Cartman who provided snow totara plants, and advised on the rooting of cuttings.
Dr. B. Molloy and the Riccarton Bush Trustees kindly allowed the collection of kahikatea seed and mycorrhizal inocula from Dean's Bush; and the Dept. of Conservation, Turangi gave permission for the collection of snow totara seed from Tongariro National Park.

I would like to thank the staff of the School of Forestry; particularly Dr. D. Norton for his introduction to New Zealand's flora, his help in the collection of snow totara scions, and his continued interest in my thesis;
Drs. D. Mead and G. Whyte for their timely advice;
Dr. R. Woollons for his help with the statistical analysis;
Messrs. K. Schasching, P. Fuller, B. Bullsmith and D. Clark for their words of wisdom and assistance; and
Miss D. Cummings for her cheerful and patient help. Special appreciation is extended to Dr. H. Billington for her help with the isozyme analysis, her advice and suggestions for sections of the manuscript, and her friendship. I would also like to thank Professor P. Bannister and Dr. M. Wilcox for their thorough reviews of sections of the manuscript.

My gratitude is extended to the staff of the Forest and Grasslands Ecology Section, Forest Research Centre for the use of their facilities. Thanks are especially due to

Dr. I. McCracken for his good advice; Mr. J. Byers for his invaluable technical assistance with the controlled environment cabinets; and Messrs. M. Lang and P. Clinton for their help with the nutrient analyses. I would like to thank Mrs. R. Young and Mr. P. Jackson of the Plant and Microbial Sciences Department for their generous help with the glasshouse work. Thanks are also due to Dr. M. Andrews and the Plant Science Department of Lincoln College for the use of their controlled environment cabinets.

Dr. C. Daugherty and his lab staff at Victoria University were very helpful in providing me with instruction and assistance in setting up an isozyme analysis lab; and I would also like to thank Mr. B. Lawson and Drs. G. Qualls and F. Sin for the loan of equipment and their advice.

For the frost experiment, Dr. I.J. Warrington of the Plant Physiology Division, DSIR was very co-operative in letting me use their facilities. I also would like to express my gratitude to Dr. D.H. Greer for all his help with the frost work and for his hospitality.

Thanks are due to my family for their continued support and encouragement during my three years away; and I especially want to thank Michael Eby for his help and support in all aspects of my work, his companionship, his good humour and his love.

Finally, I would like to thank the Association of Commonwealth Universities for their financial support over the last three years.

REFERENCES

Abbott, L.K. and A.D. Robson. 1984. The effect of mycorrhizae on plant growth. In. C.L. Powell and D.J. Bagyaraj (eds.), VA Mycorrhiza. CRC Press. Boca Raton. 113-130.

Ashton, P.S. 1969. Speciation among tropical forest trees: some deductions in the light of recent evidence. In. R.H. Lowe-McConnell (ed.), Speciation in Tropical Environments. Biol. J. Linn. Soc. 1. 155-196.

Bagyaraj, D.J. 1984. Biological interactions with VA mycorrhizal fungi. In. C.L. Powell and D.J. Bagyaraj (eds.), VA Mycorrhiza. CRC Press. Boca Raton. 131-154.

Bannister, P. 1976. Introduction to Physiological Plant Ecology. Blackwell Scientific Publications. Oxford. 273 p.

Bannister, P. 1984. The seasonal course of frost resistance in some New Zealand pteridophytes. N.Z. Jour. Bot. 22. 557-563.

Barton, I.L. 1978. Temperature and its effect on the germination and initial growth of kauri (Agathis australis) N.Z. Jour. For. Sci. 8. 327-331.

Baylis, G.T.S. 1959. Effect of vesicular-arbuscular mycorrhizas on growth of Griselinia littoralis (Cornaceae) New Phytol. 58. 274-280.

Baylis, G.T.S. 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. New Phytol. 66. 231-243.

Baylis, G.T.S. 1969. Synthesis of mycorrhizas in Podocarpus and Agathis with Endogone spores. Nature. 221. 1267-1268.

Baylis, G.T.S. 1970. Root hairs and phycomycetous mycorrhizas in phosphorus-deficient soil. Plant and Soil. 33. 713-716.

Baylis, G.T.S. 1971. Endogonaceous mycorrhizas synthesized in Leptospermum (Myrtaceae). N.Z. Jour. Bot. 9. 293-296.

Baylis, G.T.S. 1972. Fungi, phosphorus and the evolution of root systems. Search. 3 257-258.

Baylis, G.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in roots derived from it. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 373-390.

Baylis, G.T.S., R.F.R. McNabb and T.M. Morrison. 1963. The mycorrhizal nodules of podocarps. Trans. Br. Mycol. Soc. 46. 378-384.

Becking, J.H. 1965. Nitrogen fixation and mycorrhiza in Podocarpus root nodules. Plant and Soil. 23. 213-226.

Becking, J.H. 1976. Nitrogen fixation in some natural ecosystems in Indonesia. In. P.S. Nutman (ed.), Symbiotic Nitrogen Fixation in Plants. Cambridge University Press. Cambridge. 539-550.

Bergersen, F.J. and A.B. Costin. 1964. Root nodules of Podocarpus lawrencei and their ecological significance. Aust. Jour. Biol. Sci. 17. 44-48.

Bergin, D.O. and C.E. Ecroyd. 1987. Totara Provenance Trial - Seed Collection and Measurement. Project Record No. 1751. Forest Research Institute. Rotorua. 18 p.

Berry, K.M., N.B. Perry and R.T. Weavers. 1985. Foliage sesquiterpenes of Dacrydium cupressinum: identification, variation and biosynthesis. Phytochemistry. 24. 2893-2898.

Bevege, D.I. and G.D. Bowen. 1975. Endogone strain and host plant differences in development of vesicular-arbuscular mycorrhizas. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 77-86.

Beveridge, A.E. 1983. Regeneration of podocarp forests and maintenance of productivity. In. K. Thompson, A.P.H. Hodder and A.S. Edmonds (eds.), Lowland Forests in New Zealand. Univ. of Waikato. Hamilton. 93-112.

Bidwell, R.G.S. 1974. Plant Physiology. MacMillan Publishing Co. Inc. New York. 643 p.

Bieleski, R.L. 1959. Factors affecting growth and distribution of kauri (Agathis australis Salisb.). III Effect of temperature and soil conditions. Aust. J. Bot. 7. 281-288.

Boland, D.J., M.H. Brooker, G.M. Chippendale, N. Hall, B.P.M. Hyland, R.D. Johnston, D.A. Kleinig and J.D. Turner. 1984. Forest Trees of Australia. Thomas Nelson Australia and CSIRO. Melbourne. 687 p.

Bonfante-Fasolo, P. 1984. Anatomy and morphology of VA mycorrhizae. In. C.L. Powell and D.J. Bagyaraj (eds.), VA Mycorrhiza. CRC Press. Boca Raton. 5-34.

Bowen, G.D. 1980. Mycorrhizal roles in tropical plants and ecosystems. In. P. Mikola (ed.), Tropical Mycorrhizal Research. Clarendon Press. Oxford. 165-190.

Bowen, G.D., D.I. Bevege and B. Mosse. 1975. Phosphate physiology of vesicular-arbuscular mycorrhizas. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 241-260.

Brix, H. 1971. Growth responses of western hemlock and Douglas-fir seedlings to temperature regimes during day and night. Can. J. Bot. 49. 289-294.

Cameron, R.J. 1960. Natural regeneration of podocarps in the forests of the Whirinaki River valley. N.Z. Jour. For. 8. 337-354.

Cannell, M.G.R. and L.J. Sheppard. 1982. Seasonal changes in the frost hardiness of provenances of Picea sitchensis in Scotland. Forestry. 55. 137-153.

Chapin, F.S. III. 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11. 233-260.

Chapin, F.S. III, P.R. Tryon and K. van Cleve. 1983. Influence of phosphorous on growth and biomass distribution of Alaskan taiga tree seedlings. Can. Jour. For. Res. 13. 1092-1098.

Cheliak, W.M. and J.A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42. Petawawa National Forestry Institute. Canadian Forestry Service. 49 p.

Ching, K.K. and D. Bever. 1960. Provenance study of Douglas-fir in the Pacific Northwest region. Silvae Genet. 9. 11-17.

Cockayne, L. 1928. The Vegetation of New Zealand. H.R. Engelmann (J. Cramer). Weinheim/Bergstr. 364 p.

Cooper, K.M. 1975. Growth responses to the formation of endotrophic mycorrhizas in Solanum, Leptospermum, and New Zealand ferns. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 391-408.

Cooper, K.M. 1984. Physiology of VA mycorrhizal associations In. C.L. Powell and D.J. Bagyaraj (eds.), VA Mycorrhiza. CRC Press. Boca Raton. 155-186.

Copes, D.L. 1981. Isoenzyme uniformity in western red cedar seedlings from Oregon and Washington. Can. Jour. For. Res. 11. 451-453.

Cox, C.B., I.N. Healey and P.D. Moore. 1976. Biogeography, an Ecological and Evolutionary Approach. Second Edition. Blackwell Scientific Publ. Oxford. 179 p.

Crush, J.R. 1973. Significance of endomycorrhizas in tussock grassland in Otago, New Zealand. N.Z. Jour. Bot. 11. 645-660.

DSIR. 1954. General Survey of the Soils of North Island, New Zealand. Soil Bureau Bull. No. 5. 286 p. + maps.

DSIR. 1968. General Survey of the Soils of South Island, New Zealand. Soil Bureau Bull. No. 27. 404 p. + maps.

Daft, M.J. and T.H. Nicolson. 1969. Effects of Endogone mycorrhiza on plant growth. New Phytol. 68. 945-952.

Dancik, B.P. and F.C. Yeh. 1983. Allozyme variability and evolution of lodgepole pine (Pinus contorta var. latifolia) and jack pine (P. banksiana) in Alberta. Can. Jour. Genet. Cytol. 25. 57-64.

Daniels Hetrick, B.A. 1984. Ecology of VA mycorrhizal fungi. In. C.L. Powell and D.J. Bagyaraj (eds.), VA Mycorrhiza. CRC Press. Boca Raton. 57-94.

Delaunay, J. 1978. Results of Tectona grandis L.f. provenance trials six years after initiation in the Ivory Coast. In. D.G. Nikes, J. Burley and R.D. Barnes (eds.), Progress and Problems of Genetic Improvement of Tropical Forest Trees Vol. 2. Dept. of Forestry, Commonwealth Forestry Inst. University of Oxford. 734-742.

Djavanshir, K. and H. Pourbeik. 1976. Germination value - a new formula. Silvae Genet. 25. 79-82.

Doehlert, D.C. and R.B. Walker. 1981. Photosynthesis and photorespiration in Douglas-fir as influenced by irradiance, CO_2 concentration and temperature. For. Sci. 27. 641-650.

Doran, J.C. and D.J. Boland. 1984. Effects of temperature on germination of Eucalyptus microtheca. Aust. For. Res. 14. 49-55.

Dowling, T.E. and W.S. Moore. 1984. A program for estimating genetic variability within and between populations. Jour. Hered. 75. 416.

Downs, R.J. and H. Hellmers. 1975. Environment and the Experimental Control of Plant Growth. Academic Press. London. 145 p.

Dunsworth, G.B. and B.P. Dancik. 1983. Seedling variation among Alberta populations of white spruce (Picea glauca Moench) Voss) in controlled environments. Northwest Science. 57. 74-83.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons Inc. New York. 412 p.

Ewusie, J.Y. 1980. Elements of Tropical Ecology with Reference to the African, Asian, Pacific and New World Tropics. Heinemann. New York. 205 p.

Feret, P.P. 1971. Isoenzyme variation in Picea glauca (Moench)Voss seedlings. Silvae Genet. 20. 46-50.

Fins, L. and W.J. Libby. 1982. Population variation in Sequoiadendron: seed and seedling studies, vegetative propagation and isozyme variation. Silvae Genet. 31. 102-109.

Fleming, C.A. 1975. The geological history of New Zealand and its biota. In. G. Kushel (ed.), Biogeography and Ecology in New Zealand. W. Junk. The Hague. 1-86.

Florence, R.G. 1983. A perspective of the eucalypt forests; their characteristics and roles in wood production. Presented to Section H. 15th Pacific Science Congress. Dunedin. 23 p.

Forest Research Institute. 1980. Establishing Nursery-Raised Seedlings. What's New in Forest Research # 86. H.A. Holmes and Co. Ltd. Rotorua. 4 p.

Fowler, D.P. and R.W. Morris. 1977. Genetic diversity in red pine: evidence for low genic heterozygosity. Can. Jour. For. Res. 7. 343-347.

Fowler, D.P. and Y.S. Park. 1982. Range-wide black spruce provenance trials in the Maritimes. Info. Report. MFRC, Canada. No. M-X-137. 25 p.

Frakes, L.A. 1979. Climates Throughout Geologic Time. Elsevier. New York. 310 p.

Franklin, D.A. 1968. Biological flora of New Zealand 3. Dacrydium cupressinum Lamb. (Podocarpaceae) Rimu. N.Z. Jour. Bot. 6. 493-513.

Fryer, J.H. and F.T. Ledig. 1972. Microevolution of the photosynthetic temperature optimum in relation to the elevational complex gradient. Can. J. Bot. 50. 1231-1235.

Furman, T.E. 1970. The nodular mycorrhizae of Podocarpus rospigliosii. Am. Jour. Bot. 57. 910-915.

Gerdemann, J.W. 1975. Vesicular-arbuscular mycorrhizae. In. J.G. Torrey and D.T. Clarkson (eds.), The Development and Function of Roots. Academic Press. London. 575-591.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46. 235-244.

Gibbs, H.S. 1983. Development and relationships of lowland forest soils. In. K. Thompson, A.P.H. Hodder and A.S. Edmonds (eds.), Lowland Forests in New Zealand. Univ. of Waikato, Hamilton. 35-44.

Goddard, R.E. and C.A. Hollis. 1984. The genetic basis of forest tree nutrition. In. G.D. Bowen and E.K.S. Nambiar (eds.), Nutrition of Plantation Forests. Academic Press. London. 237-257.

Golomazora G.M. 1981. Light and temperature curves of photosynthesis in coniferous species. Soviet Plant Phys. 28. 183-187.

Good, R.E. and N.F. Good. 1976. Growth analysis of pitch pine seedlings under three temperature regimes. For. Sci. 22. 445-448.

Gorman, G.C. and J. Renzie. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effect of sample size. Copeia. 2. 242-249.

Gowin, T., A. Lourtioux and M. Mousseau. 1980. Influence of constant growth temperature upon the productivity and gas exchange of seedlings of Scots pine and European larch. For. Sci. 26. 301-309.

Graham, A. 1972. Outline of the origin and historical recognition of floristic affinities between Asia and eastern North America. In. A. Graham (ed.), Floristics and Paleofloristics of Asia and Eastern North America. Elsevier Pub. Co. Amsterdam. 1-16.

Greenhall, J.M. 1963. The mycorrhizal endophytes of Griselinia littoralis (Cornaceae). N.Z. Jour. Bot. 1. 389-400.

Greer, D.H. 1983. Temperature regulation of the development of frost hardiness in Pinus radiata D. Don. Aust. J. Plant Physiol. 10. 539-547.

Greer, D.H. and C.J. Stanley. 1985. Regulation of the loss of frost hardiness in Pinus radiata by photoperiod and temperature. Plant, Cell and Environment. 8. 111-116.

Greer, D.H. and I.J. Warrington. 1982. Effect of photoperiod, night temperature, and frost incidence on development of frost hardiness in Pinus radiata. Aust. J. Plant Physiol. 9. 333-342.

Griffin, A.R., E.R. Williams and K.W. Johnson. 1982. Early height growth and frost hardiness of Eucalyptus regnans provenances in twelve field trials in south-east Australia. Aust. For. Res. 12. 263-279.

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111. 1169-1194.

Grose, R.J. and W.J. Zimmer. 1958. Some laboratory germination responses of the seeds of River Red Gum, Eucalyptus camaldulensis Dehn. syn. and Eucalyptus rostrata Schlecht. Aust. Jour. Bot. 6. 129-153.

Guinon, M., J.B. Larsen and W. Spethmann. 1982. Frost resistance and early growth of Sequoiadendron giganteum seedlings of different origins. Silvae Genet. 31. 173-178.

Guries, R.P. and F.T. Ledig. 1982. Genetic diversity and population structure in pitch pine (Pinus rigida Mill). Evolution. 36. 387-402.

Hair, J.B. and E.J. Beuzenberg. 1958. Chromosomal evolution in the Podocarpaceae. Nature. 181. 1584-1586.

Hall, I.R. 1975. Endomycorrhizas of Metrosideros umbellata and Weinmannia racemosa. N.Z. Jour. Bot. 13. 463-472.

Hall, I.R. 1977. Effect of applied nutrients and endomycorrhizas on Metrosideros umbellata and Leptospermum scoparium. N.Z. Jour. Bot. 15. 481-484.

Hall, I.R. 1984. Taxonomy of VA mycorrhizal fungi. In. C.L. Powell and D.J. Bagyaraj (eds.), VA Mycorrhiza. CRC Press. Boca Raton. 57-94.

Hamrick, J.L. 1983. The distribution of genetic variation within and among natural plant populations. In S. Chambers and C. Schonewald-Cox (eds.), Genetics and Wild Population Management. Addison-Wesley. Reading. 335-348.

Hamrick, J.L., Y.B. Linhart and J.B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10. 173-200.

Harley, J.L. 1985. Specificity and penetration of tissues by mycorrhizal fungi. Proc. Indian Acad. Sci. (Plant Sci.). 94. 99-109.

Harley, J.L. and S.E. Smith. 1983. Mycorrhizal Symbiosis. Academic Press. London. 483 p.

Harwood, C.E. 1981. Frost resistance of subalpine Eucalyptus species II. Experiments using the resistance index method of damage assessment. Aust. J. Bot. 29. 209-218.

Hellmers, H. 1962. Temperature effect on optimum tree growth. In T.T. Kozlowski. Tree Growth. Ronald Press Co. New York. 275-287.

Hellmers, H. 1966. Growth response of redwood seedlings to thermoperiodism. For. Sci. 12. 277-283.

Hellmers, H., M.K. Genthe and F. Ronco. 1970. Temperature affects growth and development of Engelmann spruce. For. Sci. 16. 447-452.

Hellmers, H. and D.A. Rook. 1973. Air temperature and growth of radiata pine seedlings. N.Z. Jour. For. Sci. 3. 271-285.

Hewitt, E.J. 1966. Sand and Water Culture Methods Used in the Study of Plant Nutrition. Technical Communication No. 22. Commonwealth Agricultural Bureaux. Eastern Press Ltd. London. 547 p.

Ingestad, T. 1971. A definition of optimum nutrient requirements in birch seedlings II. Physiol. Plant. 24. 118-125.

James, I.L. 1987. Silvicultural management of the rimu forests of south Westland. FRI Bulletin No. 121. Forest Research Institute. Rotorua. 32 p.

Johnson, P.N. 1973. Mycorrhizas of Coniferous-broadleaved forest. Unpublished D. Phil. thesis. Univ. of Otago. Dunedin.

Johnson, P.N. 1976. Effects of soil phosphate level and shade on plant growth and mycorrhizas. N.Z. Jour. Bot. 14. 333-340.

Johnston, W.B. 1961. Locating the vegetation of early Canterbury: a map and the sources. Trans. Royal Soc. N.Z. Botany. 1. 5-15.

Jonsson, A., G. Eriksson and A. Franzen. 1986. Within-population variation in frost damage in Pinus contorta Dougl. seedlings after simulated autumn or late-winter conditions. Silvae Genet. 35. 96-101.

Khan, A.G. 1967. Podocarpus root nodules in sterile culture. Nature. 215. 1170.

Kimura, M. and T. Ohta. 1971. Theoretical aspects of population genetics. Princeton University Press, Princeton. 219 p.

Kobayashi, K.D., L.H. Fuchigami and C.J. Weiser. 1983. Modelling cold hardiness of red-osier dogwood. Jour. Amer. Soc. Hort. Sci. 108. 376-381.

Kormanik, P.P. and A.C. McGraw. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In. N.C. Schenck (ed.), Methods and Principles of Mycorrhizal Research. American Phytopathological Society. St. Paul. 37-46.

Kozlowski, T.T. 1971. Growth and Development of Trees. Vol. 2. Academic Press. New York. 514 p.

Kramer, P.J. 1957. Some effects of various combinations of day and night temperatures and photoperiod on the height growth of loblolly pine seedlings. For. Sci. 3. 45-53.

Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press. New York. 811 p.

Kuser, J.E. and K.K. Ching. 1980. Provenance variation in phenology and cold hardiness of western hemlock seedlings. For. Sci. 26. 463-470.

deLaubenfels, D.J. 1969. A revision of the Malesian and Pacific rainforest conifers. I. Podocarpaceae. Jour. Arnold Arboretum. 50. 274-369.

Ledig, F.T. 1987. Genetic structure and the conservation of California's endemic and near-endemic conifers. In. T.S. Elias (ed.), Conservation and Management of Rare and Endangered Plants. California Native Plant Society. Sacramento. p. 590.

Ledig, F.T. and M.T. Conkle. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey Pine (Pinus torreyana Parry ex Carr.). Evolution. 37. 79-85.

Levin, D.A. 1986. Breeding structure and genetic variation. In. M.J. Crawley (ed.), Plant Ecology. Blackwell Scientific Publications. Oxford. 217-251.

Levin, D.A. and J.B. Wilson. 1978. The genetic implications of ecological adaptations in plants. In. A.H.J. Freysen and J.W. Woldendorp (eds.), Structure and Functioning of Plant Populations. North-Holland Pub. Co. Amsterdam. 75-98.

Lewis, D.H. 1975. Comparative aspects of the carbon nutrition of mycorrhizas. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 119-148.

Linder, S. and D.A. Rook. 1984. Effects of mineral nutrition on carbon dioxide exchange and partitioning of carbon in trees. In. G.D. Bowen and E.K.S. Nambiar (eds.), Nutrition of Plantation Forests. Academic Press. London. 211-235.

Linhart, Y.B., J.B. Mitton, K.B. Sturgeon and M.L. Davis. 1981. Genetic variation in space and time in a population of ponderosa pine. Heredity. 46. 407-426.

Lundkvist, K. 1979. Allozyme frequency distributions in four Swedish populations of Norway spruce (Picea abies K.). Hereditas. 90. 127-143.

MAF. 1985. Soil-less Mixes, Bark and Sawdust For Container Grown Plants. Aglink. HPP 138: 5/3000/8/1985. Information Services, MAF, Private Bag, Wellington, N.Z. 2 p.

MacMahon, J.A. and D.C. Andersen. 1982. Subalpine forests: a world perspective with emphasis on western North America. Progress in Physical Geography. 6. 368-425.

Markham, K.R., R.F. Webby, L.A. Whitehouse, B.P.J. Molloy, C. Vilain, R. Mues. 1985. Support from flavonoid glycoside distribution for the division of Podocarpus in New Zealand. N.Z. Jour. Bot. 23. 1-13.

McEwen, W.M. 1983. Some aspects of the seed development and seedling growth of rimu, Dacrydium cupressinum Lamb. Unpublished D. Phil. thesis. Univ. of Waikato. Hamilton.

McGlone, M.S. 1985. Plant biogeography and the late Cenozoic history of New Zealand. N.Z. Jour. Bot. 23. 723-749.

McKimm, R.J. 1985. Characteristics of the wood of young and fast grown trees of Eucalyptus nitens Maiden with special reference to provenance variation. I Variations in growth, strain and density associated with provenance. Aust. For. Res. 15. 207-218.

Mead, D.J. 1984. Diagnosis of nutrient deficiencies in plantations. In. G.D. Bowen and E.K.S. Nambiar (eds.), Nutrition of Plantation Forests. Academic Press. London. 259-291.

Mengel, K. and E.A. Kirkby. 1979. Principles of Plant Nutrition. <<Der Bund>> AG. Bern. 593 p.

Menzies, M.I. 1977. Frost. In. C.G.R. Chavasse (ed). Forestry Handbook. N.Z. Inst. For. Rotorua. 92-94

Menzies, M.I., D.G. Holden, L.M. Green and D.A. Rook. 1981. Seasonal changes in frost tolerance of Pinus radiata seedlings raised in different nurseries. N.Z. Jour. For. Sci. 11. 100-111.

Mergen, F., J. Burley and G.M. Furnival. 1974. Provenance - temperature interaction in four coniferous species. Silvae Genet. 23. 200-210.

Metcalf, L.J. 1972. The Cultivation of New Zealand Trees and Shrubs. A.H. and A.W. Reed Ltd. Wellington. 292 p.

Mildenhall, D.C. 1980. New Zealand Late Cretaceous and Cenozoic plant biogeography: a contribution. Paleogeography Paleoclimatology Paleoecology. 31. 197-233.

Miller, C.N. 1977. Mesozoic conifers. The Botanical Review. 43. 217-280.

Mirams, R.V. 1951. A Study of Some of the Factors Concerned in the Natural Regeneration of the Kauri (Agathis australis). Unpublished Ph.D. thesis. Auckland Univ. Auckland. 135-136.

Mitton, J.B. 1983. Conifers. In. S.D. Tanksley and T.J. Orton (eds.), Isozymes in Plant Genetics and Breeding, Part B. Elsevier Science Publishers B.V. Amsterdam. 443-472.

Mohan, E., N. Mitchell and P. Lovell. 1984. Environmental factors controlling germination of Leptospermum scoparium (manuka). N.Z. Jour. Bot. 22. 95-100.

Molloy, B.P.J. 1969. Recent history of the vegetation. In. G.A. Knox (ed.), The Natural History of Canterbury. A.W. and A.H. Reed. Wellington. 340-360.

Moore, M.B. 1984. Seedling growth variation among Rocky Mountain populations of lodgepole pine. Silvae Genet. 33. 230-234.

Morrison, T.M. and D.A. English. 1967. The significance of mycorrhizal nodules of Agathis australis. New Phytol. 66. 245-250.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106. 283-292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S. 70. 3321-3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89. 583-590.

Nei, M. and A.K. Roychoudhury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics. 76. 379-390.

New Zealand Institute of Foresters. 1986. Forestry Handbook. Wellington North. 143 p.

New Zealand Meteorological Service. 1981. Summaries of Climatological Observations to 1980. New Zealand Meteorological Miscellaneous Publication 177. Wellington. 172 p.

Nicholson, G. 1984. Methods of Soil, Plant and Water Analysis. FRI Bulletin No. 70. Forest Research Inst. Rotorua. 24 p.

Nicolson, T.H. 1975. Evolution of vesicular-arbuscular mycorrhizas. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 25-34.

Norton, D.A., J.W. Herbert and A.E. Beveridge. 1988. The ecology of Dacrydium cupressinum, a review. N.Z. Jour. Bot. 26. 37-62.

Opeke, L.K. 1982. Tropical Tree Crops. John Wiley and Sons Ltd. New York. 312 p.

Pearce, E.A. and C.G. Smith. 1984. The World Weather Guide. Hutchinson and Co. Ltd. London. 480 p.

Perry, N.B. and R.T. Weavers. 1985. Infraspecific variation of foliage diterpenes of Dacrydium cupressinum. Phytochemistry. 24. 2233-2237.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55. 158-161.

Pollard, D.F.W. and F.T. Portlock. 1986. Intraspecific variation in stem growth of western hemlock. Can. Jour. For. Res. 16. 149-151.

Pollock, K.M., D.H. Greer and B.T. Bulloch. 1986. Frost tolerance of Acacia seedlings. Aust For. Res. 16. 337-346.

Poole, A.L. and N.M. Adams. 1980. Trees and Shrubs of New Zealand. P.D. Hasselberg, Government Printer. Wellington 257 p.

Potvin, A. 1975. A Panorama of Canadian Forests. Dept. of Envir. Canadian Forestry Service. 254 p.

Precht, H., J. Christophersen, H. Hensel, W. Larcher. 1973. Temperature and Life. Springer-Verlag. Berlin. 779 p.

Quinn, C.J. 1982. Taxonomy of Dacrydium Sol. ex Lamb. emend. deLaub. (Podocarpaceae). Aust. Jour. Bot. 30. 311-320.

Ramshaw, J.A.M., J.A. Coyne and R.C. Lewontin. 1979. The sensitivity of gel electrophoresis as a detector of genetic variation. Genetics. 93. 1019-1037.

Raven, D.H. 1973. Evolution of subalpine and alpine plant groups in New Zealand. N.Z. Jour. Bot. 11. 177-200

Redhead, J.F. 1980. Mycorrhiza in natural tropical forests. In. P. Mikola (ed.), Tropical Mycorrhizal Research. Clarendon Press. Oxford. 127-142.

Rehfeldt, G.E. 1982. Differentiation of Larix occidentalis populations from the northern Rocky Mountains. Silvae Genet. 31. 13-19.

Rehfeldt, G.E. 1983. Ecological adaptations in Douglas-fir (Pseudotsuga menziesii var. glauca) populations III Central Idaho. Can. Jour. For. Res. 13. 626-632.

Rehfeldt, G.E. and W.R. Wykoff. 1981. Periodicity in shoot elongation among populations of Pinus contorta from the northern Rocky Mountains. Ann. Bot. 48. 371-377.

Robotham, R.W., J. Lloyd and I.J. Warrington. 1978. A controlled environment room for producing advective white or black frost conditions. J. Agric. Eng. Res. 23. 301-311.

Ronghua, Y., A.F. Mark and J.B. Wilson. 1984. Aspects of the ecology of the indigenous shrub Leptospermum scoparium (Myrtaceae) in New Zealand. N.Z. Jour. Bot. 22. 483-507.

Rook, D.A., M.D. Wilcox, D.G. Holden and I.J. Warrington. 1980. Provenance variation in frost tolerance of Eucalyptus regnans F. Muell. Aust. For. Res. 10. 213-238.

Rosas, A., M. Alberdi, M. Delseny and L. Meza-Basso. 1986. A cryoprotective polypeptide isolated from Nothofagus dombeyi seedlings. Phytochemistry. 25. 2497-2500.

Roughgarden, J. 1979. Theory of Population Genetics and Evolutionary Ecology: an Introduction. MacMillan Pub. Co. Inc. New York. 634 p.

Rouse, N.G. 1986. An Investigation into Some Factors Affecting Seed Fertility and Germination in Kauri (Agathis australis). Unpublished B. For. Sci. thesis. University of Canterbury. Christchurch.

SAS. 1985. SAS/STAT™ Guide for Personal Computers, Version 6 Edition. SAS Institute Inc. Cary. 378 p.

Sa-ardavut, P., K.R. Shepherd and J.O. Awe. 1984. Effects of air and soil temperatures on growth of provenances of Eucalyptus camaldulensis Dehn. seedlings. Aust. For. Res. 14. 57-66.

St. John, T.V. and D.C. Coleman. 1983. The role of mycorrhizae in plant ecology. Can. Jour. Bot. 61. 1005-1014.

Sakagami, Y. and Y. Fujimura. 1981. Seasonal changes in the net photosynthetic and respiratory rates of Abies sachalinensis and Picea glehnii seedlings. Jour. Jap. For. Soc. 63. 194-200.

Sakai, A. and P. Wardle. 1978. Freezing resistance of New Zealand trees and shrubs. N.Z. Jour. Ecol. 1. 51-61.

Sakai, A., D.M. Paton and P. Wardle. 1979. Freezing resistance of temperate and sub-arctic conifers native to the Southern Hemisphere. Low Temp. Sci., Ser. B. 37. 107-111.

Sakai, A., D.M. Paton and P. Wardle. 1981. Freezing resistance of trees of the south temperate zone, especially subalpine species of Australia. Ecology. 62. 563-570.

Salmon, J.T. 1980. The Native Trees of New Zealand. Reed Methuen Publishers Ltd. Auckland. 384 p.

Sanders, F.E. 1975. The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. In. F.E. Sanders, B. Mosse and P.B. Tinker (eds.), Endomycorrhizas. Academic Press. London. 261-276.

Sanders, F.E., P.B. Tinker, R.L.B. Black and S.M. Palmerley. 1977. The development of endomycorrhizal root systems: I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. New Phytol. 78. 257-268.

Shaw, C.R. 1965. Electrophoretic variation in enzymes. Science. 149. 936-943.

Silen, R.R. 1962. A discussion of forest trees introduced into the Pacific Northwest. Jour. For. 60. 407-408.

Silen, R.R. 1978. Genetics of Douglas-fir. USDA For. Serv. Res. Pap. WO-35. 34 p.

Silen, R.R. and N.L. Mandel. 1983. Clinal genetic growth variation within two Douglas-fir breeding zones. Jour. For. 81. 216-220.

Silvester, W.B. and K.J. Bennett. 1973. Acetylene reduction by roots and associated soil of New Zealand conifers. Soil Biol. Biochem. 5. 171-179.

Slui, B. 1988. Genetic variation in Podocarpus totara. Unpublished B.For.Sci. thesis. University of Canterbury. Christchurch.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy; the principles and practice of numerical classification. Freeman. San Francisco. 573 p.

Sowden, J.R. 1985. Plant succession and soil development, Wanganui River catchment, South Westland, New Zealand. Unpublished M.App.Sc. thesis. Lincoln College, University of Canterbury. Christchurch.

Sporne, K.R. 1965. The Morphology of Gymnosperms, The Structure and Evolution of Primitive Seed Plants. Hutchinson University Library. London. 216 p.

Stanley, C.J. and I.J. Warrington. 1987. Seasonal frost tolerance of some ornamental, evergreen, broad-leaved and coniferous tree and shrub species. J. Amer. Soc. Hort. Sci. (in press).

Stearns, F. and J. Olson. 1958. Interactions of photoperiod and temperature affecting seed germination in Tsuga canadensis. Amer. Jour. Bot. 45. 53-58.

Steinhoff, R.J., D.G. Joyce and L. Fins. 1983. Isozyme variation in Pinus monticola. Can. Jour. For. Res. 13. 1122-1132.

Stern, K. and L. Roche. 1974. Genetics of Forest Ecosystems. Springer-Verlag. Berlin. 330 p.

Stevens, G. 1985. Lands in Collision - Discovering New Zealand's Past Geography. DSIR Information Series No. 161. Wellington. 129 p.

Suri, S.K. 1984. Analytical study of teak provenance tests in North Raipur Div. of Madhya Pradesh. Indian Forester. 110. 345-363.

Sutton, J.C. and G.L. Barron. 1972. Population dynamics of Endogone spores in soil. Can. Jour. Bot. 50. 1909-1914.

Sweet, G.B. and P.F. Wareing. 1968. A comparison of the rates of growth and photosynthesis in first-year seedlings of four provenances of Pinus contorta Dougl. Ann. Bot. 32. 735-751.

Toole, E.H., S.B. Hendricks, H.A. Borthwick and V.K. Toole. 1956. Physiology of seed germination. Ann. Rev. Plant Physiol. 7. 299-324.

Tranquillini, W. 1979. Physiological Ecology of the Alpine Timberline. Ecological Studies 31. Springer-Verlag. Berlin. 137 p.

Treshow, M. 1970. Environment and Plant Response. McGraw-Hill Book Co. 422 p.

USDA., Forest Service. 1974. Seeds of Woody Plants in the United States. U.S. Dept. of Agric. Agric. Handbook 450. Washington D.C. 883 p.

U.S. Environmental Data Service. 1968. Weather Atlas of the United States. U.S. Dept. of Commerce. Environmental Science Services Administration. Gale Research Co. Detroit. 262 p.

Van den Driessche, R. 1968. A comparison of growth responses of Douglas fir and Sitka spruce to different nitrogen, phosphorous and potassium levels in sand culture. Can. J. Bot. 46. 531-537.

Van den Driessche, R. 1973. Direct effects of nitrate and ammonium forms of nitrogen on growth and photosynthesis of slash pine seedlings. Aust. For. 36. 125-137.

Van den Driessche, R. 1978. Response of Douglas fir seedlings to nitrate and ammonium nitrogen sources at different levels of pH and iron supply. Plant and Soil. 49. 607-623.

Walker, R.B., S.P. Gessel and P.G. Haddock. 1955. Greenhouse studies in mineral requirements of conifers: Western Red Cedar. For. Sci. 1. 51-60.

Wall, A. 1923. The Riccarton Bush. Lyttleton Times Co. Christchurch. 13 p.

Wardle, P. 1964. Facets of the distribution of forest vegetation in New Zealand. N.Z. Jour. Bot. 2. 352-366.

Wardle, P. 1974. The kahikatea (Dacrycarpus dacrydioides) forest of south Westland. Proceedings of the N.Z. Ecol. Society. 21. 62-71.

Wardle, P. 1985. New Zealand timberlines 3. A synthesis. N.Z. Jour. Bot. 23. 263-271.

Warrington, I.J. and C.J. Stanley. 1987. Seasonal frost tolerance of some ornamental indigenous New Zealand plant species in the genera Astelia, Dicksonia, Leptospermum, Metrosideros, Pittosporum and Sophora. N.Z. Jour. Exp. Agric. 15. 357-365.

Warrington, I.J., D.A. Rook, D.C. Morgan and H.L. Turnbull. 1989. The influence of simulated shadelight and daylight on growth, development and photosynthesis of Pinus radiata Agathis australis, and Dacrydium cupressinum. Plant, Cell and Environment. (in press).

Webby, R.F., K.R. Markham, B.P.J. Molloy. 1987. The characterization of New Zealand Podocarpus hybrids using flavonoid markers. N.Z. Jour. Bot. 25. 355-366

Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science. 169. 1269-1278.

White. T.L., D.P. Lavender, K.K. Ching and P. Hinz. 1981. First-year height growth of southwestern Oregon Douglas-fir in three test environments. Silvae Genet. 30. 173-178.

Wilcox, M.D. and N.J. Ledgard. 1983. Provenance variation in the New Zealand species of Nothofagus. N.Z. Jour. Ecol. 6. 19-31.

Will, G.M. 1961. The mineral requirements of radiata pine seedlings. N.Z. Jour. Agric. Res. 4. 309-327.

Wolfe, J.A. 1971. Tertiary climatic fluctuations and methods of analysis of Tertiary floras. Paleogeography Paleoclimatology Paleoecology. 22. 927-957.

Wright, S. 1922. Coefficients of inbreeding and relationship. Amer. Natur. 56. 330-338.

Yeates, J.S. 1924. The root nodules of New Zealand pines. N.Z. Jour. Sci. Technol. 7. 121-124.

Yeh, F.C. and C. Layton. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine (Pinus contorta spp. latifolia). Can. Jour. Genet. Cytol. 21. 487-503.

Yeh, F.C. and D.M. O'Malley. 1980. Enzyme variations in natural populations of Douglas-fir, Pseudotsuga menziesii (Mirb.). I. Genetic variation patterns in coastal populations. Silvae Genet. 29. 83-92.

Zehnder, L.R. and F.O. Lanphaer. 1966. The influence of temperature and light on the cold hardiness of Taxus cuspidata. Proc. Am. Soc. Hortic. Sci. 89. 706-713.

Appendix A: Scientific names of species mentioned in the text in alphabetical order by common names

Common Name	Scientific Name
balsam fir	Abies balsamea (L.)Mill.
big tree	Sequoiadendron giganteum (Lindl.)Buchholz
black beech	Nothofagus solandri var. solandri (Hook.f.)Oerst.
black spruce	Picea mariana (Miller)Britton Sterns and Poggenberg
broadleaf	Griselinia littoralis Raoul
cashew	Anacardium occidentale L.
coconut palm	Cocos nucifera L.
Douglas-fir	Pseudotsuga menziesii (Mirb.)Franco.
eastern hemlock	Tsuga canadensis (L.)Carr.
Engelmann spruce	Picea engelmannii (Parry)Engelmann
European larch	Larix decidua Mill.
Hall's totara	Podocarpus hallii Kirk
hoop pine	Araucaria cunninghamii Aiton ex D.Don
jack pine	Pinus banksiana Lamb.
kahikatea	Dacrycarpus dacrydioides (A.Rich.)DeLaub.
kamahi	Weinmannia racemosa Linn.f.
karamu	Coprosma robusta Raoul
kauri	Agathis australis Salisb.
loblolly pine	Pinus taeda L.
lodgepole pine	Pinus contorta Dougl.
manuka	Leptospermum scoparium J.R.&G.Forst.
mountain beech	Nothofagus solandri var. cliffortioides (Hook.f.)Poole
Norway spruce	Picea abies (L.)Karst
oil palm	Elaeis guineensis Jacq.
pitch pine	Pinus rigida Mill.
ponderosa pine	Pinus ponderosa var. ponderosa Laws.
pygmy pine	Lepidothamnus laxifolium (Hook.f.)Quinn
radiata pine	Pinus radiata D.Don
red alder	Alnus rubra Bong.
red beech	Nothofagus fusca (Hook.f.)Oerst.
red pine	Pinus resinosa Ait.
redwood	Sequoia sempervirens (D.Don)Endl.
rimu	Dacrydium cupressinum Lamb.
river red gum	Eucalyptus camaldulensis Dehnh.
rubber	Hevea spp.
Scots pine	Pinus sylvestris L.
silver beech	Nothofagus menziesii (Hook.f.)Oerst.
Sitka spruce	Picea sitchensis (Bong.)Carr.
slash pine	Pinus elliottii var. elliottii Engelm.
snow totara	Podocarpus nivalis Hook.
southern rata	Metrosideros umbellata Cav.
teak	Tectona grandis L.f.
Torrey pine	Pinus torreyana Parry
totara	Podocarpus totara G. Benn. ex Don
western hemlock	Tsuga heterophylla (Raf.)Sarg.
western larch	Larix occidentalis Nutt.
western red cedar	Thuja plicata Donn
western white pine	Pinus monticola Dougl.
white fir	Abies concolor (Gord.&Glend.)Lindl.
white pine	Pinus strobus L.
white spruce	Picea glauca (Moench)Voss

Appendix B: Details of seed origins

Provenance	Date of Collection mo/yr	Altitude (m)	Latitude	Longitude	Grid Ref.
RIMU					
Pureora	04/85	580	38° 26′	175° 33′	N84 045675
Whirinaki	04/85	550	39° 39′	176° 41′	N95 130420
the combination of these two provenances = Central North Is.					
Oparara	04/85	210	41° 07′	172° 12′	S12 635495
Ahaura		150	42° 24′	171° 42′	
Hochstetter	02/85	260	42° 26′	171° 43′	S45 190905
Duffer's Crk.	03/85	150	42° 43′	171° 10′	S51 56N71E
Hokitika	85	30	42° 48′	170° 54′	S57 465425
Saltwater	04/85	30-90	43° 08′	170° 27′	S63 070040
Hari Hari	05/85	100	43° 10′	170° 34′	S64 167016
Jackson Bay	05/85	45	44° 03′	168° 42′	S97 558907
Waitutu	05/85	45	46° 14′	167° 15′	S175 420180
Cascade S.F.	85	no information			
Mataketake S.F.	85	no information			
Kaikohe (2 to 5 year old seedlings)	03/87	400	35° 14′	173° 45′	
KAHIKATEA					
Temperature and First Nutrition Experiments					
Whirinaki	04/86	450	38° 39′	176° 42′	
Charleston	05/85	50	41° 53′	171° 28′	K29 830230
Christchurch	03/86	10	43° 32′	172° 36′	
Isozyme Analysis and Second Nutrition Experiment					
Warkworth	04/88	60	36° 23′	174° 40′	N34 173160
Tahuna	04/87	30	37° 30′	175° 30′	N53 007817
Hamilton	04/87	45	37° 47′	175° 17′	N65 797485
Pureora	05/88	550	38° 31′	175° 32′	N93 012572
Kumara	04/87	105	42° 41′	171° 14′	S50 784536
				171° 26′	S51 935640
South Westland	04/87	120	43° 26′	170° 39′	S64 240980
				169° 58′	S70 647655
Christchurch	05/88	10	43° 32′	172° 35′	S84 970565
TOTARA					
First Nutrition Experiment					
Raglan	03/86	15	37° 47′	174° 55′	N64 458465
Invercargill	04/86	15	46° 26′	168° 15′	S181 275991
Second Nutrition Experiment					
Gwavas S.F.	87	450	39° 42′	176° 23′	
High Temperature Experiments					
Te Karaka	85	150	38° 26′	177° 49′	N88 215653
Waipoua	85	90	35° 39′	173° 33′	N18 075044
Kaikoura	85	240	42° 17′	173° 41′	S49 966056

Appendix B: Continued

Provenance	Date of Collection mo/yr	Altitude (m)	Latitude	Longitude	Grid Ref.
Totara Provenance Experiment					
Kaikohe T041	85	240	35° 21′	173° 49′	N15 333423
Waiotahi T105	85	70	38° 07′	177° 11′	N78 616060
Gisborne T059	85	50	38° 27′	177° 50′	N88 223634
Masterton T120	85	210	40° 49′	175° 38′	N158 116785
Ngaumu S.F. T129	85	370	41° 01′	175° 58′	N162 435553
Kaikoura T111	85	240	42° 17′	173° 41′	S49 966056
Peel S.F. T097	85	300	43° 54′	171° 14′	S91 770115
Frost Experiment					
Kauaeranga T029	85	30	37° 08′	175° 37′	N49 113275
Pureora T040	85	540	38° 25′	175° 35′	N84 075698
Whangamomona T128	85	210	39° 06′	174° 46′	N110 305875
New Plymouth T126	85	100	39° 07′	174° 07′	N109 690862
Taihape T125	85	490	39° 37′	175° 46′	N132 245228
Hunterville T124	85	240	39° 54′	175° 32′	N139 035900
Pohangina T123	85	150	40° 09′	175° 50′	N144 315615
			40° 08′	175° 51′	N144 331637
Otaki T122	85	30	40° 47′	175° 10′	N157 695825
			40° 47′	175° 08′	N157 660822
Isozyme Analysis					
Warkworth	04/88	30	36° 23′	174° 40′	N34 173160
Owhango	04/88	440	39° 00′	175° 23′	N101 885004
TOTARA VAR. WAIHOENSIS					
Lafontaine R.	04/88	45	43° 07′	170° 33′	S64 155070
Lk. Matheson	04/88	70	43° 27′	169° 55′	S70 600643
HALL'S TOTARA					
Whakapapa	04/88	595	39° 06′	175° 49′	N112 293870
Ohakune	04/88	975	39° 21′	175° 28′	N121 977577
SNOW TOTARA					
High Temperature Experiments					
Mt. Cheeseman	85	1220	43° 10′	171° 40′	S66 160020
Whakapapa	05/88	1220	39° 12′	175° 34′	N112 060758
Hanmer	05/88	1320	42° 29′	172° 51′	S47 212833
Temple Basin Lo	04/88	915	42° 55′	171° 33′	S59 054324
Temple Basin Hi	04/88	1370	42° 55′	171° 34′	S59 069324
Isozyme Analysis					
Whakapapa	04/88	1250	39° 12′	175° 35′	N112 070760
Jack's Pass	01/88	915	42° 28′	172° 49′	S47 192840
Temple Basin	01/88	950	42° 54′	171° 34′	S59 053326
Hooker Valley	02/88	715	43° 43′	170° 05′	S79 760320
Rob Roy Valley	02/88	1200	44° 28′	168° 44′	S114 595390
PYGMY PINE					
Whakapapa	04/88	1250	39° 12′	175° 35′	N112 070760

Appendix C: Sums of squares for analyses of variance comparing measurements of growth for individual species in the first nutrition experiment

Model for Totara:	SOURCE OF VARIATION	df
Whole Plot	Nutrient Treatment	5
	Replicate	1
	Error A	5
Split Plot	Provenance	1
	Treatment X Provenance	5
	Error B	54
	TOTAL	71

	SS	df	p > F
RIMU			
TOTAL WEIGHT			
	0.04898	5	0.079
	0.00001	1	0.989
	0.01240	5	0.119
	0.01473	2	0.007
	0.01313	10	0.469
	0.06399	48	
ROOT WEIGHT			
	0.00084	5	0.780
	0.00009	1	0.623
	0.00174	5	0.045
	0.00228	2	0.001
	0.00165	10	0.334
	0.00677	48	
ROOT WEIGHT/TOTAL WEIGHT			
	0.18688	5	0.013
	0.00500	1	0.073
	0.01894	5	0.040
	0.01390	2	0.014
	0.01957	10	0.249
	0.07143	48	
HEIGHT			
	11567.4	5	0.007
	17.5104	1	0.765
	880.177	5	0.525
	483.059	2	0.323
	2351.71	10	0.362
	10014.5	48	
DIAMETER			
	0.23278	5	0.092
	0.03010	1	0.186
	0.06427	5	0.571
	0.15003	2	0.016
	0.17975	10	0.391
	0.79437	48	
LEAF WEIGHT			
	0.01863	5	0.033
	0.00007	1	0.747
	0.00301	5	0.142
	0.00319	2	0.015
	0.00287	10	0.599
	0.01656	48	
STEM WEIGHT			
	0.00323	5	0.020
	0.00001	1	0.824
	0.00041	5	0.387
	0.00035	2	0.109
	0.00057	10	0.672
	0.00831	48	
LEAF WEIGHT/TOTAL WEIGHT			
	0.09691	5	0.042
	0.00167	1	0.185
	0.01763	5	0.005
	0.00368	2	0.148
	0.01426	10	0.153
	0.04436		
NUMBER OF BRANCHES			
	29.9028	5	0.063
	2.04167	1	0.272
	6.70833	5	0.382
	28.5555	2	0.001
	10.4444	10	0.591
	59.50	48	

Appendix C: Continued

KAHIKATEA

	SS	df	p > F
TOTAL WEIGHT			
	1.42974	5	0.001
	0.00060	1	0.758
	0.02851	5	0.354
	0.05277	2	0.009
	0.04279	10	0.581
	0.24067	48	
ROOT WEIGHT			
	0.07727	5	0.001
	0.00045	1	0.312
	0.00180	5	0.797
	0.00954	2	0.004
	0.00837	10	0.390
	0.03693	48	
ROOT WEIGHT/TOTAL WEIGHT			
	0.40441	5	0.001
	0.00029	1	0.676
	0.00358	5	0.819
	0.00111	2	0.714
	0.01867	10	0.351
	0.07837	48	
HEIGHT			
	138262.4	5	0.001
	24.0000	1	0.821
	2116.50	5	0.299
	1396.78	2	0.137
	4364.09	10	0.261
	16188.1	48	
DIAMETER			
	6.02156	5	0.001
	0.01260	1	0.605
	0.20677	5	0.160
	0.29444	2	0.005
	0.19764	10	0.632
	1.19000	48	
LEAF WEIGHT			
	0.31786	5	0.001
	0.00000	1	0.990
	0.00566	5	0.447
	0.00794	2	0.042
	0.00551	10	0.901
	0.05623	48	
STEM WEIGHT			
	0.13188	5	0.001
	0.00001	1	0.914
	0.00305	5	0.093
	0.00197	2	0.047
	0.00297	10	0.469
	0.01449	48	
LEAF WEIGHT/TOTAL WEIGHT			
	0.13308	5	0.003
	0.00001	1	0.989
	0.00677	5	0.501
	0.00349	2	0.329
	0.01311	10	0.582
	0.07379	48	
NUMBER OF BRANCHES			
	5825.07	5	0.023
	66.6667	1	0.542
	777.833	5	0.024
	16.7361	2	0.857
	646.847	10	0.316
	2592.25	48	

Appendix C: Continued

	SS	df	p > F
TOTARA			
TOTAL WEIGHT			
	3.70883	5	0.004
	0.06472	1	0.193
	0.23550	5	0.293
	0.03685	1	0.324
	0.10237	4	0.603
	1.81970	49	
ROOT WEIGHT			
	0.10207	5	0.162
	0.00387	1	0.230
	0.01162	5	0.498
	0.00198	1	0.389
	0.00912	4	0.490
	0.12878	49	
ROOT WEIGHT/TOTAL WEIGHT			
	0.22593	5	0.029
	0.00622	1	0.129
	0.03418	5	0.034
	0.00671	1	0.614
	0.00663	4	0.769
	0.14124	49	
HEIGHT			
	91487.1	5	0.001
	107.188	1	0.677
	1564.79	5	0.764
	61.3407	1	0.752
	1127.46	4	0.763
	29849.7	49	
DIAMETER			
	3.47587	5	0.001
	0.00456	1	0.749
	0.13023	5	0.707
	0.00091	1	0.886
	0.03323	4	0.943
	2.15925	49	
LEAF WEIGHT			
	1.10656	5	0.005
	0.02026	1	0.164
	0.07627	5	0.207
	0.01444	1	0.239
	0.03385	4	0.511
	0.49818	49	
STEM WEIGHT			
	0.33327	5	0.001
	0.00248	1	0.346
	0.01175	5	0.517
	0.00074	1	0.605
	0.00449	4	0.801
	0.13456	49	
LEAF WEIGHT/TOTAL WEIGHT			
	0.09122	5	0.019
	0.00018	1	0.737
	0.01121	5	0.225
	0.00081	1	0.473
	0.00129	4	0.932
	0.07611	49	
NUMBER OF BRANCHES			
	616.065	5	0.001
	6.86992	1	0.289
	20.1267	5	0.646
	1.66667	1	0.600
	19.9944	4	0.510
	293.500	49	

Appendix D: Linear components of the main analyses of variance in the first nutrition experiment

	Total Weight	Leaf Weight	Root Weight	Height	Diameter
RIMU					
R^2	0.29	0.39	0.04	0.44	0.09
Intercept	0.0828	0.0337	0.0366	41.844	0.8733
Slope	0.0001	0.0001	0.00002	0.0617	0.0002
F of Slope	0.0001	0.0001	0.0442	0.0001	0.0054
KAHIKATEA					
R^2	0.80	0.73	0.41	0.81	0.68
Intercept	0.1520	0.0442	0.0813	57.943	1.2056
Slope	0.0007	0.0003	0.0001	0.2139	0.0014
F of Slope	0.0001	0.0001	0.0001	0.0001	0.0001
TOTARA					
R^2	0.56	0.59	0.22	0.70	0.47
Intercept	0.2558	0.1096	0.1167	37.882	1.2546
Slope	0.0013	0.0007	0.0002	0.2224	0.0012
F of Slope	0.0001	0.0001	0.0001	0.0001	0.0001

Appendix E: A comparison of the anomalous totara seedlings with the normal totara seedlings in the first nutrition experiment

	10 ppm Treatment		60 ppm Treatment	
	Normal Seedlings	Abnormal Seedlings	Normal Seedlings	Abnormal Seedlings
Height (mm)	23.7	83.8	39.7	102.5
Total (g) Weight	0.1590	0.6763	0.2425	0.6356
Element Concentrations (%)				
Nitrogen	0.5535	0.8475	0.7370	0.8950
Phosphorous	0.0450	0.0910	0.0870	0.1920
Potassium	0.9560	0.9560	1.1120	1.2750
Magnesium	0.2370	0.2500	0.2320	0.2270
Calcium	1.0000	1.1700	0.9700	1.0300

Appendix F: Sums of squares from analysis of variance of the net photosynthetic rates measured in the first nutrition experiment

SOURCE OF VARIATION	SS	df	p > F
Nutrient Treatment	0.03348	2	0.001
Species	0.00347	2	0.279
Treatment X Species	0.00309	4	0.668
Error	0.03498	27	
TOTAL		35	

Appendix G: Sums of squares from the analyses of variance of whole-tree nutrient concentrations in the first nutrition experiment

Model:	SOURCE OF VARIATION	df
	Nutrient Treatment	5
	Species	2
	Treatment X Species	10
	Error	18
	TOTAL	35

Element	SS	df	p > F
NITROGEN	1.47124	5	0.0001
	0.68179	2	0.0001
	0.05241	10	0.0001
	0.00843	18	
PHOSPHORUS	0.08853	5	0.0001
	0.03965	2	0.0001
	0.02515	10	0.0001
	0.00032	18	
POTASSIUM	0.99893	5	0.0001
	0.55961	2	0.0001
	0.10299	10	0.0001
	0.01069	18	
CALCIUM	0.14775	5	0.0001
	0.23260	2	0.0001
	0.05652	10	0.0001
	0.00748	18	
MAGNESIUM	0.00260	5	0.0001
	0.03549	2	0.0001
	0.00805	10	0.0001
	0.00047	18	

Appendix H: Sums of squares for analyses of variance comparing measurements of growth of three species in the second nutrition experiment

Model:	SOURCE OF VARIATION	df
	Species	2
	Nutrient Treatment	2
	Mycorrhiza Treatment	1
	Species X Nutrient	4
	Size Class	4
	Error	66
	TOTAL	79

	SS	df	p > F
TOTAL WEIGHT			
	0.11846	2	0.001
	0.05127	2	0.001
	0.00454	1	0.074
	0.03908	4	0.001
	0.02723	4	0.002
	0.09062	66	
ROOT WEIGHT			
	0.02188	2	0.001
	0.00603	2	0.001
	0.00020	1	0.469
	0.00725	4	0.002
	0.00702	4	0.002
	0.02496	66	
ROOT WEIGHT/TOTAL WEIGHT			
	0.36119	2	0.001
	0.03221	2	0.035
	0.00110	1	0.625
	0.03505	4	0.118
	0.06212	4	0.014
	0.30185	66	
HEIGHT			
	2307.00	2	0.001
	8526.49	2	0.001
	1674.45	1	0.001
	3924.53	4	0.001
	1842.31	4	0.012
	8669.42	66	
DIAMETER			
	1.65130	2	0.001
	0.35843	2	0.001
	0.09453	1	0.001
	0.11682	4	0.001
	0.23379	4	0.001
	0.33884	66	

	SS	df	p > F
LEAF WEIGHT			
	0.01048	2	0.001
	0.00143	2	0.008
	0.02114	1	0.001
	0.00787	4	0.001
	0.00243	4	0.018
	0.01252	66	
STEM WEIGHT			
	0.00216	2	0.001
	0.00024	2	0.016
	0.00500	1	0.001
	0.00124	4	0.001
	0.00072	4	0.002
	0.00257	66	
LEAF WEIGHT/TOTAL WEIGHT			
	0.47133	2	0.001
	0.04808	2	0.001
	0.00429	1	0.176
	0.02333	4	0.048
	0.02678	4	0.028
	0.15150	66	
NUMBER OF BRANCHES			
	414.571	2	0.001
	27.8808	2	0.001
	3.61250	1	0.047
	10.7275	4	0.024
	12.0855	4	0.013
	58.5103	66	
PERCENT INFECTION			
	153.750	2	0.745
	1236.65	2	0.100
	47531.2	1	0.001
	2417.52	4	0.065
	898.576	4	0.489
	17131.0	66	

Appendix I: Sums of squares for analyses of variance comparing measurements of growth for individual species in the second nutrition experiment

Model:	SOURCE OF VARIATION	df
	Nutrient Treatment	2
	Mycorrhiza Treatment	1
	Nutrient X Mycorrhiza	2
	Size Class	4
	Error	20
	TOTAL	29

	SS	df	p > F
RIMU			
TOTAL WEIGHT			
	0.00044	2	0.004
	0.00053	1	0.001
	0.00024	2	0.034
	0.00075	4	0.002
	0.00060	20	
ROOT WEIGHT			
	0.00005	2	0.039
	0.00010	1	0.001
	0.00007	2	0.015
	0.00016	4	0.003
	0.00014	20	
ROOT WEIGHT/TOTAL WEIGHT			
	0.00273	2	0.726
	0.02168	1	0.034
	0.03505	2	0.031
	0.07026	4	0.013
	0.08422	20	
HEIGHT			
	396.800	2	0.001
	381.633	1	0.001
	205.867	2	0.011
	251.533	4	0.025
	358.867	20	
DIAMETER			
	0.02217	2	0.136
	0.06533	1	0.002
	0.00617	2	0.551
	0.06950	4	0.026
	0.10050	20	
LEAF WEIGHT			
	0.00012	2	0.004
	0.00009	1	0.002
	0.00004	2	0.085
	0.00012	4	0.021
	0.00016	20	
STEM WEIGHT			
	0.000012	2	0.021
	0.000010	1	0.012
	0.000003	2	0.326
	0.000022	4	0.012
	0.000026	20	
LEAF WEIGHT/TOTAL WEIGHT			
	0.00951	2	0.230
	0.00112	1	0.548
	0.01362	2	0.129
	0.02134	4	0.172
	0.06001	20	
NUMBER OF BRANCHES			
	0.80000	2	0.056
	0.53333	1	0.048
	0.26667	2	0.349
	0.80000	4	0.197
	2.40000	20	
PERCENT INFECTION			
	740.000	2	0.169
	20280.0	1	0.001
	740.000	2	0.169
	353.333	4	0.761
	3806.67	20	

Appendix I: Continued

	SS	df	p > F
KAHIKATEA			
TOTAL WEIGHT			
	0.01573	2	0.001
	0.00252	1	0.011
	0.00037	2	0.574
	0.00380	4	0.045
	0.00643	20	
ROOT WEIGHT			
	0.00110	2	0.014
	0.00033	1	0.088
	0.00011	2	0.588
	0.00100	4	0.080
	0.00205	20	
ROOT WEIGHT/TOTAL WEIGHT			
	0.05462	2	0.001
	0.00016	1	0.805
	0.01014	2	0.158
	0.01410	4	0.266
	0.04998	20	
HEIGHT			
	3857.40	2	0.001
	504.300	1	0.001
	14.6000	2	0.794
	488.467	4	0.017
	625.533	20	
DIAMETER			
	0.16350	2	0.001
	0.01875	1	0.045
	0.00350	2	0.659
	0.09083	4	0.004
	0.08217	20	
LEAF WEIGHT			
	0.00325	2	0.001
	0.00037	1	0.004
	0.00019	2	0.096
	0.00030	4	0.123
	0.00072	20	
STEM WEIGHT			
	0.00143	2	0.001
	0.00016	1	0.005
	0.00004	2	0.339
	0.00027	4	0.012
	0.00032	20	
LEAF WEIGHT/TOTAL WEIGHT			
	0.04089	2	0.001
	0.00086	1	0.225
	0.00452	2	0.032
	0.00212	4	0.449
	0.01101	20	
NUMBER OF BRANCHES			
	22.4667	2	0.001
	4.80000	1	0.036
	1.40000	2	0.491
	3.80000	4	0.431
	19.0000	20	
PERCENT INFECTION			
	1860.00	2	0.063
	17280.0	1	0.001
	1860.00	2	0.063
	1086.00	4	0.466
	5833.33	20	

Appendix I: Continued

	SS	df	p > F
TOTARA			
TOTAL WEIGHT			
	0.07831	2	0.012
	0.00202	1	0.558
	0.00089	2	0.922
	0.04514	4	0.162
	0.05497	10	
ROOT WEIGHT			
	0.01308	2	0.046
	0.00004	1	0.874
	0.00031	2	0.904
	0.01156	4	0.190
	0.01537	10	
ROOT WEIGHT/TOTAL WEIGHT			
	0.01603	2	0.121
	0.01672	1	0.041
	0.00845	2	0.294
	0.01772	4	0.287
	0.03046	10	
HEIGHT			
	8196.82	2	0.003
	924.800	1	0.151
	1275.82	2	0.237
	3324.57	4	0.146
	3830.18	10	
DIAMETER			
	0.28958	2	0.001
	0.01800	1	0.193
	0.00492	2	0.772
	0.11499	4	0.066
	0.09251	10	

	SS	df	p > F
LEAF WEIGHT			
	0.01498	2	0.003
	0.00159	1	0.153
	0.00077	2	0.577
	0.00536	4	0.168
	0.00665	10	
STEM WEIGHT			
	0.00196	2	0.009
	0.00013	1	0.333
	0.00015	2	0.576
	0.00114	4	0.136
	0.00126	10	
LEAF WEIGHT/TOTAL WEIGHT			
	0.02618	2	0.017
	0.01849	1	0.014
	0.00381	2	0.434
	0.01947	4	0.128
	0.02102	10	
NUMBER OF BRANCHES			
	15.3417	2	0.077
	0.05000	1	0.885
	0.40833	2	0.915
	17.9015	4	0.177
	22.8485	10	
PERCENT INFECTION			
	1054.17	2	0.119
	10125.0	1	0.001
	1054.17	2	0.119
	1152.65	4	0.288
	1989.02	10	

Appendix J: Sums of squares for analyses of variance comparing measurements of photosynthetic rate for three species in the second nutrition experiment

Model:	SOURCE OF VARIATION	df
	Nutrient Treatment	2
	Mycorrhiza Treatment	1
	Nutrient X Mycorrhiza	2
	Error	30
	TOTAL	35

Species	SS	df	p > F	Species	SS	df	p > F
RIMU				KAHIKATEA			
	6.76581	2	0.003		34.6868	2	0.001
	6.57238	1	0.001		37.6975	1	0.001
	3.40850	2	0.039		0.67795	2	0.629
	14.1305	30			21.6491	30	
TOTARA							
	40.8832	2	0.001				
	10.3360	1	0.001				
	6.60029	2	0.014				
	13.9488	22					

Appendix K: Sums of squares from the analyses of variance of whole-tree nutrient concentrations in the second nutrition experiment

Model:	SOURCE OF VARIATION	df
	Species	2
	Nutrient Treatment	2
	Mycorrhiza Treatment	1
	Nutrient X Mycorrhiza	2
	Error	10
	TOTAL	17

Element	SS	df	p > F
NITROGEN	0.10785	2	0.549
	1.98890	2	0.002
	0.00114	1	0.910
	0.05937	2	0.713
	0.84753	10	
PHOSPHORUS	0.01851	2	0.005
	0.02552	2	0.002
	0.01687	1	0.005
	0.00339	2	0.235
	0.01009	10	

Appendix L: Sums of squares for the analyses of variance in the germination experiment

Source of Variation	Rimu SS	Rimu df	Rimu p > F	Kahikatea SS	Kahikatea df	Kahikatea p > F
Temperature	525.89	11	0.0001	744.31	11	0.0001
Replicate	29.78	2	0.0040	5.87	2	0.6354
Error	45.55	22		139.46	22	
TOTAL		35			35	

Appendix M: Sums of squares for analyses of variance of measured growth variables in the first temperature experiment

Model:

SOURCE OF VARIATION	df
Species	2
Temperature Treatment	3
Size Class	4
Species X Size Class	8
Error	42
TOTAL	59

	SS	df	p > F
TOTAL WEIGHT			
	14.63707	2	0.0001
	0.95774	3	0.0001
	0.95886	4	0.0001
	0.50776	8	0.0522
	1.2416	42	
ROOT WEIGHT			
	0.86473	2	0.0001
	0.02888	3	0.0001
	0.04435	4	0.0001
	0.01435	8	0.0439
	0.03375	42	
LEAF WEIGHT/TOTAL WEIGHT			
	0.40357	2	0.0001
	0.00776	3	0.0290
	0.00150	4	0.7492
	0.00277	8	0.8885
	0.03282	42	
ROOT WEIGHT/TOTAL WEIGHT			
	0.14388	2	0.0001
	0.03787	3	0.0001
	0.00484	4	0.1976
	0.00512	8	0.5776
	0.03221	42	
NUMBER OF BRANCHES			
	899.266	2	0.0001
	77.127	3	0.0003
	30.285	4	0.0700
	24.240	8	0.4953
	135.519	42	
LEAF WEIGHT			
	5.93557	2	0.0001
	0.31126	3	0.0003
	0.29690	4	0.0009
	0.20702	8	0.0717
	0.54663	42	
STEM WEIGHT			
	0.29920	2	0.0001
	0.07233	3	0.0001
	0.05207	4	0.0001
	0.02368	8	0.1177
	0.07154	42	
STEM WEIGHT/TOTAL WEIGHT			
	0.13182	2	0.0001
	0.01918	3	0.0001
	0.00270	4	0.0399
	0.00194	8	0.4585
	0.01028	42	
HEIGHT			
	15470.82	2	0.0001
	10592.98	3	0.0001
	3330.98	4	0.0001
	491.39	8	0.7532
	4137.68	42	
NUMBER OF LEAVES			
	19589436.6	2	0.0001
	2586510.8	3	0.0001
	757813.6	4	0.0019
	612332.0	8	0.0613
	1555629.9	42	

Appendix N: Sums of squares for analyses of variance of measured growth variables for individual species in the second temperature experiment

Model:	SOURCE OF VARIATION	df
Whole Plot	Temperature Treatment	3
	Light Treatment	2
	Error A	6
Split Plot	Size Class	4
	Species X Light	8
	Error B	30
	TOTAL	53

RIMU

TOTAL WEIGHT SS	df	p > F	LEAF WEIGHT SS	df	p > F
0.05941	3	0.008	0.02092	3	0.003
0.00022	2	0.944	0.00003	2	0.968
0.01136	6	0.715	0.00253	6	0.830
0.15794	4	0.000	0.03009	4	0.001
0.04751	8	0.091	0.01156	8	0.170
0.09210	30		0.02728	30	

ROOT WEIGHT SS	df	p > F	STEM WEIGHT SS	df	p > F
0.00442	3	0.005	0.00283	3	0.044
0.00014	2	0.571	0.00012	2	0.737
0.00069	6	0.724	0.00111	6	0.562
0.01329	4	0.001	0.01213	4	0.001
0.00366	8	0.040	0.00318	8	0.125
0.00573	30		0.00678	30	

LEAF WEIGHT/TOTAL WEIGHT SS	df	p > F	STEM WEIGHT/TOTAL WEIGHT SS	df	p > F
0.13491	3	0.001	0.01640	3	0.015
0.00678	2	0.196	0.00146	2	0.571
0.00545	6	0.831	0.00399	6	0.789
0.00920	4	0.345	0.00834	4	0.194
0.01971	8	0.305	0.00383	8	0.927
0.05908	30		0.03849	30	

ROOT WEIGHT/TOTAL WEIGHT SS	df	p > F	HEIGHT SS	df	p > F
0.17553	3	0.001	9209.91	3	0.034
0.00691	2	0.299	357.82	2	0.728
0.01357	6	0.561	3212.99	6	0.445
0.01073	4	0.435	12324.58	4	0.002
0.01495	8	0.705	4892.95	8	0.367
0.08246	30		16105.93	30	

Appendix N: Continued

SS	df	p > F
NUMBER OF BRANCHES		
45.0340	3	0.128
2.4276	2	0.801
31.6879	6	0.430
51.8447	4	0.063
70.4217	8	0.138
154.9083	30	
DIAMETER		
1.06689	3	0.001
0.02606	2	0.376
0.06758	6	0.810
1.06405	4	0.001
0.13860	8	0.644
0.68858	30	

SS	df	p > F
LEAF AREA		
413.856	3	0.011
0.495	2	0.983
88.379	6	0.618
515.080	4	0.001
297.057	8	0.101
593.628	30	

SS	df	p > F
KAHIKATEA		
TOTAL WEIGHT		
3.82810	3	0.001
0.06068	2	0.574
0.29877	6	0.928
3.14384	4	0.003
1.00842	8	0.623
5.49423	30	
ROOT WEIGHT		
0.18952	3	0.001
0.00897	2	0.014
0.00288	6	0.999
0.14797	4	0.004
0.04777	8	0.655
0.27372	30	
LEAF WEIGHT/TOTAL WEIGHT		
0.04926	3	0.222
0.00263	2	0.803
0.05037	6	0.240
0.01213	4	0.729
0.03988	8	0.577
0.20265	30	
ROOT WEIGHT/TOTAL WEIGHT		
0.03650	3	0.051
0.00991	2	0.197
0.01554	6	0.513
0.00574	4	0.740
0.02001	8	0.559
0.09889	30	

SS	df	p > F
LEAF WEIGHT		
0.840855	3	0.011
0.020659	2	0.719
0.177760	6	0.737
0.679885	4	0.019
0.319358	8	0.612
1.710544	30	
STEM WEIGHT		
0.42574	3	0.001
0.00454	2	0.584
0.02308	6	0.922
0.32746	4	0.001
0.07333	8	0.638
0.40923	30	
STEM WEIGHT/TOTAL WEIGHT		
0.04990	3	0.033
0.00302	2	0.501
0.01729	6	0.265
0.01209	4	0.251
0.01363	8	0.611
0.07283	30	
HEIGHT		
77553.65	3	0.001
1726.77	2	0.330
3859.64	6	0.634
30156.23	4	0.001
16822.41	8	0.039
30280.89	30	

Appendix N: Continued

	SS	df	p > F
NUMBER OF BRANCHES			
	13134.68	3	0.044
	863.37	2	0.629
	5170.41	6	0.484
	11432.07	4	0.028
	8718.46	8	0.339
	31371.51	30	
DIAMETER			
	7.32093	3	0.001
	0.18077	2	0.234
	0.29081	6	0.794
	3.47884	4	0.001
	1.14863	8	0.187
	3.21283	30	
LEAF AREA			
	35773.69	3	0.002
	1167.49	2	0.443
	3745.28	6	0.769
	21010.21	4	0.004
	9659.76	8	0.415
	38820.59	30	
TOTARA			
TOTAL WEIGHT			
	3.32826	3	0.206
	0.15850	2	0.866
	3.22400	6	0.243
	7.02956	4	0.007
	1.41535	8	0.788
	7.38233	30	
LEAF WEIGHT			
	0.95796	3	0.215
	0.07041	2	0.808
	0.95617	6	0.206
	1.66020	4	0.014
	0.42544	8	0.748
	2.02207	30	
ROOT WEIGHT			
	0.14622	3	0.142
	0.00217	2	0.943
	0.11086	6	0.358
	0.11455	4	0.161
	0.04330	8	0.893
	0.31196	30	
STEM WEIGHT			
	0.24865	3	0.260
	0.01057	2	0.897
	0.28727	6	0.239
	1.06229	4	0.001
	0.14949	8	0.706
	0.65181	30	
LEAF WEIGHT/TOTAL WEIGHT			
	0.00536	3	0.240
	0.00310	2	0.403
	0.00582	6	0.731
	0.00452	4	0.607
	0.01548	8	0.278
	0.03267	30	
STEM WEIGHT/TOTAL WEIGHT			
	0.02322	3	0.034
	0.00342	2	0.203
	0.00814	6	0.273
	0.02784	4	0.001
	0.00498	8	0.657
	0.01977	30	
ROOT WEIGHT/TOTAL WEIGHT			
	0.00647	3	0.360
	0.00132	2	0.582
	0.01002	6	0.259
	0.01709	4	0.023
	0.02787	8	0.015
	0.02366	30	
HEIGHT			
	14118.92	3	0.226
	1193.16	2	0.790
	14624.32	6	0.441
	57779.61	4	0.002
	10521.45	8	0.727
	47804.83	30	

Appendix N: Continued

	SS	df	p > F		SS	df	p > F
NUMBER OF BRANCHES				LEAF AREA			
	108.728	3	0.218		15250.11	3	0.133
	25.179	2	0.537		1348.37	2	0.706
	109.497	6	0.071		10982.20	6	0.401
	384.697	4	0.000		15062.26	4	0.101
	203.902	8	0.010		5364.09	8	0.854
	156.052	30			33561.49	30	
DIAMETER							
	0.95019	3	0.029				
	0.20021	2	0.224				
	0.30913	6	0.922				
	2.15263	4	0.032				
	0.47640	8	0.882				
	3.28809	30					
KAURI							
TOTAL WEIGHT				LEAF WEIGHT			
	0.19964	3	0.035		0.05194	3	0.049
	0.01032	2	0.664		0.00261	2	0.711
	0.07067	6	0.054		0.02165	6	0.047
	0.06038	4	0.032		0.02034	4	0.019
	0.06290	8	0.174		0.02111	8	0.118
	0.16046	30			0.04747	30	
ROOT WEIGHT				STEM WEIGHT			
	0.00735	3	0.008		0.01885	3	0.044
	0.00045	2	0.430		0.00100	2	0.683
	0.00138	6	0.249		0.00742	6	0.044
	0.00080	4	0.324		0.00625	4	0.028
	0.00278	8	0.065		0.00349	8	0.546
	0.01706	30			0.01594	30	
LEAF WEIGHT/TOTAL WEIGHT				STEM WEIGHT/TOTAL WEIGHT			
	0.02520	3	0.031		0.01583	3	0.006
	0.00629	2	0.329		0.00259	2	0.366
	0.00845	6	0.792		0.00257	6	0.909
	0.02815	4	0.056		0.00822	4	0.188
	0.02229	8	0.441		0.02065	8	0.070
	0.08741	30			0.04005	30	
ROOT WEIGHT/TOTAL WEIGHT				HEIGHT			
	0.01011	3	0.173		7672.32	3	0.005
	0.00144	2	0.565		768.14	2	0.222
	0.00865	6	0.353		1180.18	6	0.762
	0.00793	4	0.200		2220.48	4	0.207
	0.01004	8	0.449		1138.58	8	0.911
	0.03984	30			11341.48	30	

Appendix N: Continued

SS	df	p > F
LEAF AREA		
853.05	3	0.024
8.07	2	0.911
256.19	6	0.140
301.51	4	0.029
382.73	8	0.083
777.99	30	
DIAMETER		
1.18668	3	0.010
0.03027	2	0.707
0.24717	6	0.432
0.23562	4	0.239
0.52123	8	0.162
1.29711	30	
MOUNTAIN BEECH		
TOTAL WEIGHT		
7.12598	3	0.020
1.30936	2	0.217
1.97217	6	0.946
7.76339	4	0.220
11.57498	8	0.363
24.68851	30	
LEAF WEIGHT		
1.35879	3	0.010
0.10868	2	0.368
0.27464	6	0.952
1.40299	4	0.141
2.15118	8	0.220
3.59202	30	
ROOT WEIGHT		
0.12933	3	0.060
0.01931	2	0.430
0.05943	6	0.891
0.12553	4	0.356
0.08258	8	0.917
0.53944	30	
STEM WEIGHT		
1.93907	3	0.023
0.51894	2	0.144
0.57099	6	0.926
1.73065	4	0.273
3.14092	8	0.316
6.22046	30	
LEAF WEIGHT/TOTAL WEIGHT		
0.16691	3	0.047
0.03648	2	0.084
0.06836	6	0.160
0.02807	4	0.392
0.03824	8	0.659
0.12988	30	
STEM WEIGHT/TOTAL WEIGHT		
0.05761	3	0.100
0.02143	2	0.072
0.03504	6	0.189
0.01921	4	0.288
0.01942	8	0.703
0.07136	30	
ROOT WEIGHT/TOTAL WEIGHT		
0.03914	3	0.041
0.00371	2	0.586
0.01484	6	0.629
0.00858	4	0.643
0.05066	6	0.121
0.06752	30	
LEAF AREA		
35607.25	3	0.015
84.01	2	0.972
8717.45	6	0.892
26050.84	4	0.204
44800.30	8	0.253
79569.02	30	
DIAMETER		
2.70656	3	0.239
0.54051	2	0.602
2.93426	6	0.339
1.50195	4	0.465
2.81484	8	0.554
8.04797	30	

Appendix O: Sums of squares of net photosynthetic rates of seedlings in the second temperature experiment

Species	SS Temperature Treatment (3 df)	p > F	SS Error (28 df)
RIMU	0.03061	0.0001	0.02247
KAHIKATEA	0.00813	0.0001	0.00337
TOTARA	0.05426	0.0001	0.01165
KAURI	0.01356	0.0001	0.01153
MOUNTAIN BEECH	0.22886	0.0001	0.09189

Appendix P: Recipe for half-strength Hoagland's solution (from Plant Physiology Div., DSIR)

Chemical Source	Concentration (g/l)
$Ca(NO_3)_2$ X $4H_2O$	0.59038
KNO_3	0.25278
$MgSO_4$ X $7H_2O$	0.24648
KH_2PO_4	0.06804
10% DTPA NaFe	0.02080
KCl	0.00315
H_3BO_3	0.00143
$MnCl_2$ X $4H_2O$	0.00091
$ZnSO_4$ X $7H_2O$	0.00011
$CuSO_4$ X $5H_2O$	0.00004
Na_2MoO_4 X $2H_2O$	0.00001

Appendix Q: Sums of squares from the analyses of variance of measures of growth of snow totara cuttings in the preliminary third temperature trial

Model:	SOURCE OF VARIATION	df
	Temperature	2
	Error	9
	TOTAL	11

	SS	df	p > F
INCREASE IN TOTAL BRANCH L.			
	68343158.0	2	0.0002
	12669109.0	9	
	81012267.0	11	
INCREASE IN TOTAL # BRANCHES			
	86410.2	2	0.0006
	20134.7	9	
	106544.9	11	
INCREASE IN DIAMETER			
	4.943	2	0.0014
	1.487	9	
	6.431	11	

	SS	df	p > F
% INCREASE IN TOTAL BRANCH L.			
	151734.2	2	0.0003
	29181.1	9	
	180915.3	11	
% INCREASE IN TOTAL # BRANCHES			
	400363.3	2	0.0001
	25124.7	9	
	425487.9	11	
% INCREASE IN DIAMETER			
	1881.7	2	0.0006
	456.7	9	
	2338.5	11	

Appendix R: Sums of squares from the analyses of variance of measures of growth and photosynthesis of snow totara cuttings in the third temperature experiment

Model:	SOURCE OF VARIATION	df
	Temperature	1
	Provenance	3
	Size Class	8
	Error	101
	TOTAL	113

	SS	df	p > F		SS	df	p > F
TOTAL WEIGHT				LEAF WEIGHT			
	0.02717	1	0.017		0.00474	1	0.091
	0.10490	3	0.001		0.03203	3	0.001
	0.42038	8	0.001		0.09504	8	0.001
	0.46222	101			0.16494	101	
ROOT WEIGHT				STEM WEIGHT			
	0.00702	1	0.001		0.00015	1	0.424
	0.00691	3	0.001		0.00750	3	0.001
	0.03128	8	0.001		0.02979	8	0.001
	0.03803	101			0.02310	101	
LEAF WEIGHT/TOTAL WEIGHT				STEM WEIGHT/TOTAL WEIGHT			
	0.01224	1	0.022		0.00694	1	0.013
	0.10289	3	0.001		0.02943	3	0.001
	0.10083	8	0.001		0.04179	8	0.001
	0.22983	101			0.10899	101	
ROOT WEIGHT/TOTAL WEIGHT				HEIGHT			
	0.03760	1	0.001		3.16667	1	0.903
	0.02577	3	0.039		4290.26	3	0.001
	0.02119	8	0.527		15007.4	8	0.001
	0.30026	101			21651.3	101	
NUMBER OF BRANCHES				DIAMETER			
	3.50877	1	0.257		0.00219	1	0.859
	24.1262	3	0.035		0.61857	3	0.035
	65.9156	8	0.004		1.69078	8	0.004
	272.309	101			6.99968	101	
PHOTOSYNTHESIS							
Model:	Source of Variation						
	Temperature				4.56690	1	0.001
	Species				5.07999	1	0.001
	Provenance				11.2519	5	0.001
	Error				38.1096	112	

Appendix S: Sums of squares from the analyses of variance of measured growth variables of the seven totara provenances

Model:	SOURCE OF VARIATION	df
	Provenance	6
	Size class	6
	Error	34
	TOTAL	46

	SS	df	p > F
DIFFERENCE IN TOTAL WEIGHT			
	61.5277	6	0.654
	75.2340	6	0.540
	500.7868	34	
DIFFERENCE IN ROOT WEIGHT			
	4.7646	6	0.171
	3.0828	6	0.411
	16.6324	34	
DIFFERENCE IN HEIGHT			
	36522.98	6	0.407
	86242.29	6	0.041
	195886.73	34	
DIFFERENCE IN NUMBER OF FIRST ORDER BRANCHES			
	298.828	6	0.914
	562.076	6	0.706
	5061.971	34	
DIFFERENCE IN LEAF WEIGHT			
	17.1458	6	0.663
	17.2163	6	0.661
	141.9240	34	
DIFFERENCE IN STEM WEIGHT			
	7.1379	6	0.727
	11.1791	6	0.479
	67.3122	34	
DIFFERENCE IN DIAMETER			
	9.6939	6	0.034
	3.4977	6	0.479
	21.0561	34	
DIFFERENCE IN NUMBER OF SECOND ORDER BRANCHES			
	7094.6	6	0.257
	4833.5	6	0.486
	29461.1	34	

Appendix T: Values of intercepts (b_0) and slopes (b_1) calculated in the regressions of the change in the ratio of growth variables (warm/cool) over the duration of the totara provenance experiment

b_0	p>[T]	b_1	p>[T]	b_0	p>[T]	b_1	p>[T]
TOTAL WEIGHT							
T041				T059			
0.9999	0.0001	-0.0003	0.9131	1.3037	0.0001	-0.0048	0.0886
T097				T105			
0.9217	0.0001	0.0031	0.3304	1.0705	0.0001	-0.0013	0.5480
T111				T120			
0.9904	0.0001	0.0004	0.8740	0.9598	0.0001	0.0051	0.2103
T129							
1.2405	0.0001	-0.0011	0.7781				
LEAF WEIGHT							
T041				T059			
0.9692	0.0001	0.0006	0.8163	1.2824	0.0001	-0.0037	0.1593
T097				T105			
0.9241	0.0002	0.0053	0.0216	1.0557	0.0001	-0.0012	0.5797
T111				T120			
1.0205	0.0001	0.0013	0.7043	0.9703	0.0008	0.0067	0.2149
T129							
1.2448	0.0001	0.0002	0.9560				
ROOT WEIGHT							
T041				T059			
1.0515	0.0001	-0.0017	0.4865	1.3837	0.0001	-0.0083	0.0457
T097				T105			
0.9472	0.0001	0.0003	0.9115	1.1500	0.0001	-0.0045	0.1368
T111				T120			
0.9231	0.0001	-0.0017	0.5644	0.9445	0.0002	0.0040	0.3703
T129							
1.3064	0.0001	-0.0041	0.4141				
HEIGHT							
T041				T059			
1.0170	0.0001	-0.0027	0.1312	0.9924	0.0001	-0.0004	0.7893
T097				T105			
0.9888	0.0001	-0.0005	0.6967	1.0959	0.0001	-0.0023	0.0865
T111				T120			
1.0802	0.0001	-0.0022	0.0108	0.8996	0.0001	-0.0001	0.9410
T129							
1.0819	0.0001	-0.0023	0.0607				

Appendix T: Continued

b_0	p>[T]	b_1	p>[T]	b_0	p>[T]	b_1	p>[T]
DIAMETER							
T041				T059			
1.0268	0.0001	-0.0006	0.7326	1.2289	0.0001	-0.0036	0.1156
T097				T105			
0.9377	0.0001	0.0002	0.8481	1.0225	0.0001	0.0005	0.7360
T111				T120			
0.9637	0.0001	-0.0004	0.8247	1.0217	0.0001	0.0031	0.0603
T129							
1.0788	0.0001	-0.0005	0.7251				

Appendix U: Details of the recipes for extraction, gel and electrode buffers, voltages, enzyme names and stain solutions used in the isozyme analysis

---

SEED EXTRACTION BUFFER
(adapted from Cheliak and Pitel, 1984)
used with rimu seed

10 ml Tris-citrate buffer (pH 7)
0.4 mM NAD
0.2 mM NADP
0.001 M Ascorbic acid
0.001 M EDTA (disodium)
100 mg Cleland's reagent

Adjust pH to 6.8 - 7.5 with 0.1 M Tris and add 5 drops of beta-mercaptoethanol.

EXTRACTION BUFFER II
(adapted from Feret, 1971)
used with seed of all totara species, totara foliage and kahikatea embryos

50 mls 0.1M Tris
0.1 g Ascorbic acid
0.047 g Cysteine
8.55 g Sucrose
0.5 ml Tween
1 ml MgCl (10%)
1 ml CaCl (10%)
2 drops beta-mercaptoethanol

Adjust pH to 7.5 with HCl.

GEL AND ELECTRODE BUFFERS AND APPROPRIATE VOLTAGES AND STAINS

Poulik Gel Buffer - 300 volts - stain for AAT, ACP, ADH, GDH, PEP, PGI, 6PGD, SDH in rimu
pH 8.7 with concentrated HCl

for 2 l: 18.42 g Tris
2.1 g Citric acid

Poulik Electrode Buffer
pH 8.2

for 2 l: 37.1 g Boric acid
4.8 g Sodium hydroxide

Amine Citrate Gel Buffer - 170 volts - stain for ACON, LAP MDH, ME, IDH, G6PGD in rimu
pH 6.0 with N-(3Aminopropyl)morpholine

for 2 l: 0.841 g Citric acid

Appendix U: Continued

Amine Citrate Electrode Buffer
pH 6.1 with N-(3Aminopropyl)morpholine

for 2 l: 16.8 g Citric acid

Ridgeway Gel Buffer - 50 mA - stain for AAT, ACP, DIA, PEP, 6PG, PGI, PGM in totara and kahikatea
pH 8.1 with LiOH

for 2 l: 5.036 g Lithium hydroxide
37.1 g Boric acid

Ridgeway Electrode Buffer
pH 8.5

for 2 l: 20 ml Ridgeway electrode buffer
7.62 g Tris
2.1 g Citric acid

Histadine Gel Buffer - 150 V - stain for ADH, ADH, G6P, IDH, LAP, MDH, ME, SDH, SKDH in totara and kahikatea
pH 7.0 with 1.0M Citric acid

for 2 l: 30.27 g Tris

Histadine Electrode Buffer
pH 7.0 with 1.0M Tris

for 2 l: 4.2 g L-Histidine
0.16 g EDTA

STAIN BUFFERS

I 24.2 g Tris + 1 l distilled water pH 8 with HCl

II 100 ml Stain Buffer I + 400 ml distilled water

III 99 ml Ridgeway Gel Buffer + 1 ml Ridgeway Electrode Buffer

= 1 l distilled water
10 ml electrode buffer
3.635 g Tris
1.05 g citric acid
pH 8.5

= 1 l distilled water
2.52 g LiOH
18.55 g H_3BO_3
pH 8.1

IV 15.7 g Tris + 9.03 g citric acid + 1 l distilled water pH 7

V 27.2 g Na acetate + 1 l distilled water pH 5

VI 15.6 g NaH_2PO_4 + 1 l distilled water pH 7

Appendix U: Continued

ENZYME NAMES AND STAINING SOLUTIONS

For Rimu

AAT - Aspartate aminotransferase
50 ml Stain Buffer I
200 mg DL-aspartic acid
100 mg alpha-ketoglutaric acid
1 mg pyroxidal-5'-phosphate
300 mg Fast Blue BB salt

ACP - Acid phosphatase
50 ml Stain Buffer V
50 mg alpha-napthyl acid phosphatase
50 mg Black K salt
0.25 ml 10% $MgCl_2$

ACON - Aconitase dehydrogenase
50 ml Stain Buffer I
70 mg cis-aconitic acid
20 u isocitrate dehydrogenase
30 mg NBT
25 mg NADP
10 mg $MgCl_2$
2 mg PMS

ADH - Alcohol
50 ml Stain Buffer III
10 ml 95% ethanol
20 mg NAD
10 mg NBT
5 mg PMS

GDH - Glutamate dehydrogenase
50 ml Stain Buffer II
2 g L-glutamic acid
5 mg PMS
5 mg NAD
10 mg NBT

IDH - Isocitrate dehydrogenase
50 ml Stain Buffer II
200 mg DL-isocitric acid
5 mg NADP
10 mg NBT
5 mg PMS
10 mg $MgCl_2$

LAP - Leucine aminopeptidase
50 ml Stain Buffer IV
10 mg L-leucyl-beta-napthylamide
10 mg $MgCl_2$
50 mg Black K salt

MDH - Malate dehydrogenase
50 ml Stain Buffer III
350 mg malic acid
5 mg NAD
10 mg NBT
5 mg PMS

ME - Malic enzyme
50 ml Stain Buffer III
350 mg malic acid
10 mg $MgCl_2$
5 mg PMS
5 mg NADP
10 mg MTT

PGI - Phosphoglucose isomerase
50 ml Stain Buffer III
25 mg Na-fructose-6-phosphate
10 u G6PDH
5 mg NADP
10 mg NBT
5 mg PMS
10 mg $MgCl_2$

Appendix U: Continued

PEP - Peptidase
50 ml Stain Buffer II
50 mg 0-dianisidine
80 mg DL-leucyl-L-alanine
80 mg L-leucylglycyl-glycine
80 mg L-phenylalanyl-L-proline
80 mg L-leucyl-L-tyrosine
4 mg peroxidase
10 mg L-amino acid oxidase
50 mg $MgCl_2$

G6PDH - Glucose-6-phosphate dehydrogenase
50 ml Stain Buffer II
200 mg D-glucose-6-phosphate
5 mg PMS
5 mg NADP
10 mg NBT
10 mg $MgCl_2$

6PGD - 6-phosphogluconate dehydrogenase
50 ml Stain Buffer II
20 mg Ba-6-phospho-gluconic acid
5 mg NADP
10 mg MTT
5 mg PMS
10 mg $MgCl_2$

SDH - Sorbitol dehydrogenase
50 ml Stain Buffer I
125 mg D-sorbitol
2.5 mg MTT
5 mg NAD
10 mg NBT
5 mg PMS

For Kahikatea and Totara

AAT
25 ml 0.2M Tris pH 8.0
25 ml AAT substrate solution
= 500 ml Distilled water
14.2 g Na phosphate (di)
5.0 g PVP-40T
1.33 g L-Aspartic acid
0.5 g EDTA
0.365 g Alpha-Ketoglutaric acid
1 ml Fast blue BB (10%)

ACP
45 ml 0.2M Na acetate pH 5
1 ml MgCl (10%)
150 mg Na-alpha-napthyl acid phosphate
75 mg Fast Garnet GBC salt

ADH
40 ml 0.2M Tris pH 8.0
10 mg NAD
10 mg PMS
10 mg NBT
10 ml 95% Ethanol

DIA - Diaphorase
50 ml 0.2M Tris pH 8.0
1 mg 2,6-dichlorophenol
12.5 mg NADH
10 mg MTT

GDH
45 ml 0.2M Tris pH 8.0
400 mg L-glutamic acid phosph.
30 mg NAD
10 mg PMS
10 mg MTT
10 mg NBT

G6P
50 ml 0.2M Tris pH 8.0
200 mg D-glucose-6-
20 mg NADP
10 mg MTT
10 mg PMS
10 mg MgCl

IDH
46 ml 0.2M Tris pH 8.0
200 mg DL-isocitric acid
10 mg NADP
10 mg PMS
10 mg MgCl

LAP
50 ml Stain Buffer IV
10 mg L-leucyl-beta-napthylamide
25 mg Black K salt
10 mg MgCl

Appendix U: Continued

MDH
same as for rimu

ME
same as for rimu

PEP
5 ml 0.2M Tris pH 8.0
45 ml Distilled water
60 mg L-leucyl-glycyl glycine
60 mg Glycyl-L-leucine
60 mg L-leucyl-1-alanine
10 mg Peroxidase
10 mg Amino acid oxidase
10 mg MgCl

6PG
20 ml 0.2M Tris pH 8.0
10 mg 6-phosphogluconic acid
40 mg NADP
60 mg PMS
40 mg MTT
40 mg MgCl

PGI
45 ml 0.2M Tris pH 8.0
25 mg Fructose-6-phosphate phosphate
10 mg NADP
10 mg PMS
10 mg MTT
10 mg G-6-PDH
10 mg MgCl

PGM - Phosphoglucomutase
45 ml 0.2M Tris pH 8.0
300 mg Glucose-1-

10 mg NADP
10 mg MTT
10 mg PMS
10 mg MgCl
50 u G-6-PDH
0.1 mg Glucose-1,6 diP

SDH
50 ml 0.2M Tris pH 8.0
250 mg D-sorbitol
10 mg NAD
10 mg NBT
8
5 mg PMS

SKDH - Shikimic dehydrogenase
20 ml 0.2M Tris pH 8.0
0.3 g Agar boil, stir
add to 20 ml 0.2M Tris pH

10 mg Shikimic acid
10 mg PMS
10 mg NBT
10 mg NADP

For Totara foliage
MR - Menadione reductase
50 ml Tris pH 8.0
17 mg Menadione
17 mg NADH
10 mg NBT

Appendix V: Sums of squares of analyses of variance for the actual frost damage scores and the arcsin transformation of the percentage of damage

Model: For totara provenances

	SOURCE OF VARIATION	df
Whole Plot	Temperature Treatment	2
	Replicate	1
	Error A	2
Split Plot	Provenance	7
	Provenance X Replicate	7
	Treatment X Provenance	16
	Treatment X Replicate X Provenance	14
	Error B	48
	TOTAL	97

	Actual Score			Arcsin Transformation		
	SS	df	p > F	SS	df	p > F
TOTARA						
	158.7616	2	0.0001	36703.69	2	0.0001
	3.0817	1	0.0010	827.38	1	0.0010
	1.5410	2	0.0130	533.95	2	0.0217
	29.3425	7	0.0001	9637.93	7	0.0001
	0.2051	7	0.9880	72.08	7	0.9917
	14.4849	16	0.0001	9066.36	16	0.0001
	1.4160	14	0.8305	525.33	14	0.8642
	7.7630	48		3084.85	48	

Model: For rimu and kahikatea provenances

	SOURCE OF VARIATION	df
Whole Plot	Temperature Treatment	2
	Replicate	1
	Error A	2
Split Plot	Provenance	6
	Species	1
	Provenance X Replicate	6
	Treatment X Provenance	12
	Treatment X Species	2
	Treatment X Replicate X Provenance X Species	12
	Error B	42
	TOTAL	86

Appendix V: Continued

	Actual Score			Arcsin Transformation		
	SS	df	p > F	SS	df	p > F
RIMU AND KAHIKATEA						
	310.515	2	0.0007	100629.52	2	0.0006
	0.118	1	0.700	55.48	1	0.550
	0.212	2	0.443	62.47	2	0.690
	6.442	6	0.0001	3576.53	6	0.0001
	5.457	1	0.0001	2613.06	1	0.0001
	0.428	6	0.759	435.14	6	0.526
	13.230	12	0.0001	7681.19	12	0.0001
	10.872	2	0.0001	5786.75	2	0.0001
	0.465	12	0.985	509.00	12	0.898
	5.355	42		3510.61	42	